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Synthesis and Evaluation of Novel Iminosugars as Potential Male Contraceptive Agents; and the Chemistry of 2,3-Dihydropyridin-4-(1*H*)-ones and Related Enaminones in Multicomponent Reactions

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Abstract

Synthesis and Evaluation of Novel Iminosugars as Potential Male Contraceptive Agents; and the Chemistry of 2,3-Dihydropyridin-4-(1*H*)-ones and Related Enaminones in Multicomponent Reactions

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The University of Kansas, 2010

The iminosugar *N*-butyl-1-deoxynojirimycin (*n*B-DNJ) has reversible, non-hormonal contraceptive effects on C57B/L6 mice at micromolar concentrations. In order to increase the potency and bioavailability of this lead compound, a series of novel iminosugars was synthesized as inhibitors of two potential target enzymes – *ceramide-specific glucosyltransferase* (CGT) and *β-glucosidase 2* (GBA2). The new derivatives were shown to be inactive as inhibitors of CGT and are awaiting testing in a GBA2 assay. Efforts were made to identify additional protein targets of the iminosugars by preparing two iminosugar affinity labels that were used to isolate a potential new iminosugar target.

A method was developed to functionalize 2,3-dihydropyridin-4(1*H*)-ones by taking advantage of the nitrogen-induced nucleophilicity of the beta-carbon (C5) of the enamine moiety. Reaction of 2,3-dihydropyridin-4(1*H*)-ones with aliphatic and aromatic aldehydes under acidic conditions furnished 5,5'-(methylene)bis(2,3-dihydropyridin-4(1*H*)-ones). In the presence of the reducing agent triethylsilane, the same reaction provided C5 alkylated derivatives. This

chemistry was extended to 4-(pyrrolidin-1-yl)furan-2(5*H*)-one, an enaminone with an exocyclic nitrogen. A three-component Mannich aminomethylation of 2,3-dihydropyridin-4(1*H*)-ones and 4-(pyrrolidin-1-yl)furan-2(5*H*)-one, carbamates, and formaldehyde was achieved when lithium perchlorate was present in the reaction mixture. This chemistry was extended to the reaction of exocyclic enaminones with formaldehyde and malonates to furnish the corresponding methylmalonates. Mechanist studies suggest that this reaction proceeds via the formation of 2-methylenemalonates (Knoevenagel condensation), which is followed by a nucleophilic (Michael) addition of the enaminone to the methylenemalonates. The methylmalonate reaction products were cyclized under acidic conditions to form bicyclic lactams (octahydroquinoline-3-carboxylates and cyclopenta[*b*]pyridine-3-carboxylates). Oxidation of the octahydroquinoline-3-carboxylates furnished 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylates, a class of compounds known to possess ionotropic properties. In a related reaction, 3-aminocyclohex-2-enones, formaldehyde and methyl cyanoacetate directly furnished the corresponding bicyclic 2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitriles. In this case the reaction was catalyzed by a phosphine, which promoted both, the Knoevenagel reaction and the bicyclic lactam formation.

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The last five years of my life have been filled with highs and lows that come with being a graduate student. I am thankful to many people who stood by my side through this journey. It would not have been possible without the support from all of them.

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List of abbreviations

Ac – acetate

Ak – alkyl

Ar – aryl

BLK – blank

Bn – benzyl

br – broad

Boc – *tert*-butoxycarbonyl

BSA – bovine serum albumin

Bu – butyl

CGT – ceramide-specific glucosyltransferase

CHF – congestive heart failure

d – doublet

DCC – 1,3-dicyclohexylcarbodiimide

DCE – dichloroethane

DCM – dichloromethane

DIBAL-H – diisobutylaluminum hydride

DIAD – diisopropyl azodicarboxylate

DMAP – 4-*N*,*N*-dimethylaminopryidine

DMF – *N*,*N*-dimethylformamide

EDCI – 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

EtOAc – ethyl acetate

EtOH – ethanol

GBA2 – *β-glucosidase* 2

GSLs – glycosphigolipids

GlcCer – glucosylceramide

HATU – *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate

HPLC – high pressure liquid chromatography

IBX – 2-iodoxybenzoic acid

IOOC – intramolecular oxime-olefin cycloaddition

IR – infrared

J – coupling constant

LAH – lithium aluminum hydride

L-Selectride – lithium tri-*sec*-butylborohydrate

m – multiplet

MCR – multi-component reaction

Me – methyl

MeCN – acetonitrile

MeOH – methanol

μM – micromolar

MUG – 4-methylumbelliferyl-β-D-glucuronide

NBD – N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl

*n*B-DNJ – *N*-butyl-1-deoxynojirimycin

*n*N-DGJ – *N*-nonyl-1-deoxygalactonojirimycin

*n*N-6-Me-DGJ – *N*-nonyl-1,6-dideoxygalactonojirimycin

NMM – *N*-methylmorpholine

NMO – *N*-methylmorpholine oxide

NMR – nuclear magnetic resonance

NOESY – nuclear overhauser enhancement spectroscopy

PDE – phosphodiesterase

PKB – protein kinase B

s – singlet

SAR – structure/activity relationship

sulfo-SBED – sulfo-*N*-hydroxysuccinimidyl-2-(6-[biotinamido]-2-(p-azido-benzamido)-hexanoamido) ethyl-1,3'-dithioproprionate

SDS – sodium dodecylsulfate

t – triplet

TBAF – tetrabutylammonium fluoride

TBDPS – *tert*-butyldiphenylsilyl

TBS – *tert*-butyldimethylsilyl

TEA – triethylamine

TES – triethylsilyl

TESOTf – triethylsilyl triflate

$Ti(O\textit{i}Pr)_4$ – titanium tetraisopropoxide

TLC – thin layer chromatography

TMS – trimethylsilyl

THF – tetraydrofuran

UV – ultraviolet

Chapter 1

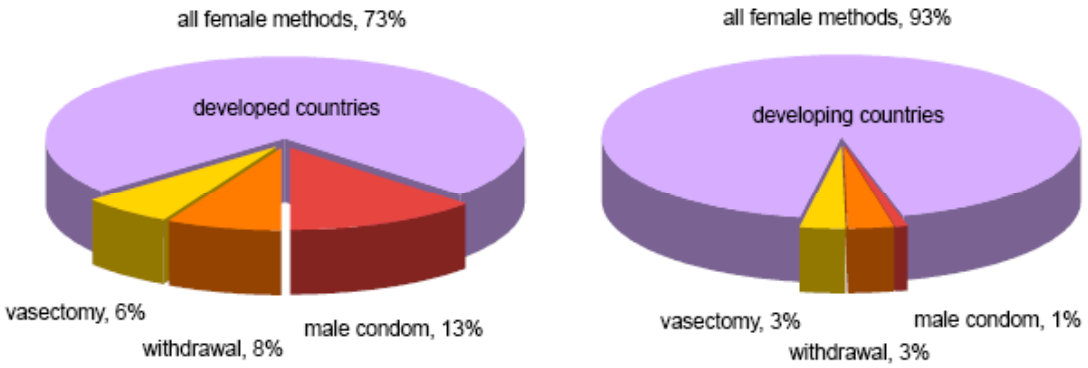
Synthesis and Evaluation of Novel Iminosugars as Potential Male Contraceptive Agents

1.1 Background

Use of modern contraceptives benefits women and their partners in many ways. Access to contraceptives allows couples to make responsible decisions and to reduce unintended pregnancies, unplanned births and abortions.[2] Despite a negative stereotype of men being irresponsible regarding birth control, a positive attitude toward male contraception has been acknowledged on a cross-cultural basis.[3] However, compared to the broad availability of female options, methods of male contraception are limited (**Figure 1**).[1]

Among the most common existing male methods, withdrawal is known as unreliable;[4] condoms are often not accepted as a long-term contraceptive method, for long-term use is asscociated with a relatively high failure rate.[5] As an

Figure 1. Use of male contraceptives in developed and developing countries, respectively.[1]



alternative method, vasectomy has significant drawbacks, because the reversal procedure (vasovasectomy) is costly and effective in achieving pregnancy in only ~50% of cases.[6,7] Male hormonal contraceptives are often linked to side effects caused by synthetic androgen in the regimen, including acne, muscle gain and cardiovascular risks.[8-10] To develop a new, safe, effective and reversible male contraceptive is clearly a great need. Given the high cost of development, biological approaches (vaccines, antibodies, proteins, etc.) are less likely to be commercially viable in the market compared to small molecule therapeutics. Therefore, the strategy is to focus on obtaining small molecules that interact with targets present in the male reproductive system, which could potentially lead to a male contraceptive agent.

Yet, there are obstacles in the path to discovering an ideal male contraceptive. First of all, its efficacy should be at least equal to that of the current female oral contraceptives. In order to meet this requirement, a male contraceptive has to be potent enough to inactivate 250×10^6 sperms in one ejaculate, while female hormonal agents only need to suppress ovulation of one or a few occytes. Secondly, an ideal “male pill” is supposed to take action without displaying adverse effects. And lastly, a good pharmacokinetic profile is necessary to deliver the contraceptive to its site of action, in particular, to traverse the blood-testis barrier.

In 2002, van der Spoel *et al*. reported that an alkylated iminosugar, *N*-butyl 1-deoxynojirimycin (*n*B-DNJ) achieved reversible infertility in male mice.[11] The success of this non-hormonal small molecule as a male contraceptive agent

suggested that the hurdles listed previously were not insurmountable with this class of compounds.

Iminosugars, first isolated and synthesized in the 1960's,[12,13] differ from monosaccharides in that the endocyclic oxygen atom is replaced by a basic nitrogen. They are widespread in plants and microorganisms and are often inhibitors of glycosidases. Iminosugars structurally resemble the sugar moiety of the natural enzyme substrates (**Figure 2**).[14] Since the protonated nitrogen atom mimicks the positive charge of the oxocarbenium transition state of the natural substrate, in the enzyme catalytic process, the iminosugar is incorporated into the active site of the glycosidase and inhibits enzymatic function.

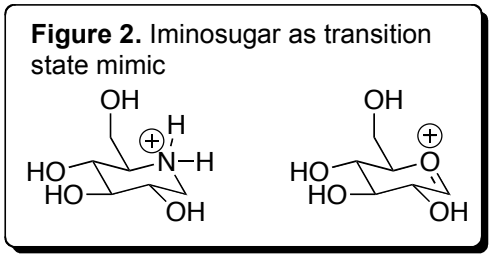


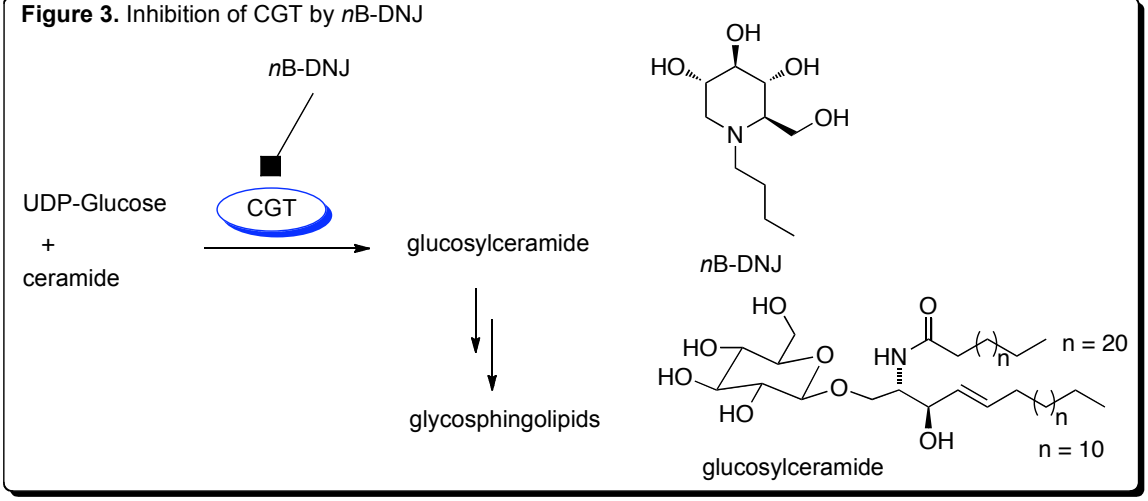
Figure 2. Iminosugar as transition state mimic

1.1.1 CGT as target of *n*B-DNJ

The *N*-alkylated iminosugar, *n*B-DNJ (Miglustat, Zavesca®), is used to treat glycosphingolipids (GSLs) storage disorders such as Gaucher's disease.[15] The pharmacological mechanism of action involves the inhibition of *ceramide-specific glucosyltransferase* (CGT),[16,17] a key enzyme that occupies a bridging position in the GSLs metabolic pathway, and connects glucose with complex lipids. GSLs are also abundant in sperm[18,19] and appear to be crucial in the process of spermatogenesis, as male mice deficient in enzymes of the GSLs

biosynthetic pathway are infertile.[20,21] Thus, the GSLs biosynthetic pathway is thought to provide a good target for small molecule-induced male fertility control, mainly via the inhibition of CGT, as depicted in **Figure 3**.

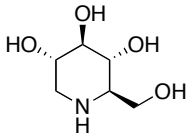
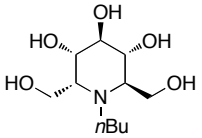
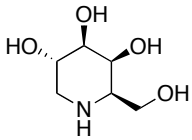
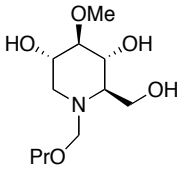
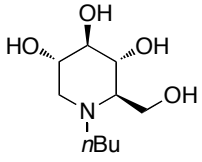
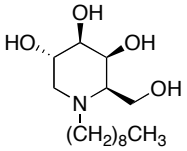
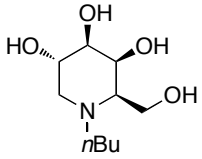
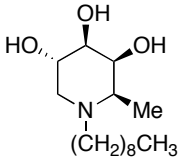
Figure 3. Inhibition of CGT by *n*B-DNJ



As demonstrated in van der Spoel's research, C57BL/6 mice treated with *n*B-DNJ showed reversible inhibition of fertility, starting 3 weeks after daily oral administration of 15 mg/kg. Fertility returned 4 weeks after cessation of administration. Based upon histological analysis, the major effect of *n*B-DNJ is malformation of the acrosomal cap, sperm head and the mitochondrial sheath. The reproductive hormones including LH, FSH and testosterone are not affected by *n*B-DNJ at that dose. Body weight, testicular weight and mating behavior are not altered either. Supported by these results, *n*B-DNJ appeared to be a promising lead compound toward the development of male contraceptives for humans. However, a recent study involving five human subjects failed to show any impact on sperm concentration, motility, morphology and the ability to undergo the acrosome reaction, after treatment with 100 mg of *n*B-DNJ twice daily for 6 weeks.[22] It was also reported that it had no apparent effect on

spermatogenesis in rabbits, as well as in other strains of mice.[23] The mechanism underlying the species specificity remains largely unknown, although it could be partially attributed to the relatively poor potency of *n*B-DNJ against CGT (IC_{50} = ~20 μM), and the short half-life, a result of its hydrophilicity. With improved potency and pharmacokinetic profile, iminosugars may still hold promise as candidates for male fertility control. Structure-activity relationship (SAR) information is usually pivotal, in order to achieve such improvements. Yet, not many *n*B-DNJ analogues have been tested as CGT inhibitors, and neither is adequate structure-activity relationship (SAR) data available. **Table 1** summarizes the available data.

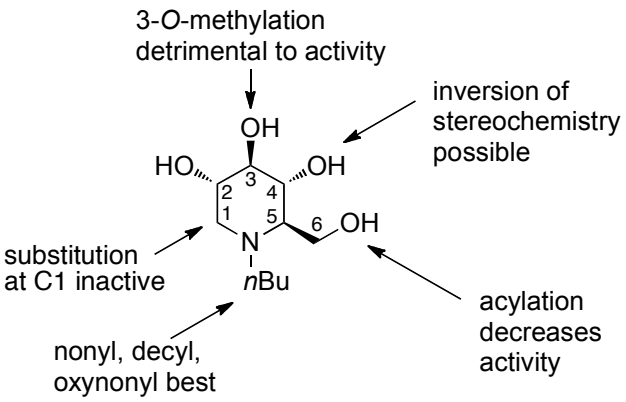
Table 1. Inhibitory activities of iminosugars on CGT from literature[24,25]

Structure	Name	CGT IC_{50} (μM)	Structure	Name	CGT IC_{50} (μM)
	DNJ	> 200*		*N*-butyl-α-homo-nojirimycin	> 200*
	DGJ	> 200*		*N*-7-oxadecyl-3-*O*-methyl-DNJ	> 200*
	*n*B-DNJ	20.4		*n*N-DGJ	10.6
	*n*B-DGJ	30		*n*N-6-Me-DGJ	400

* no inhibition at 200 μM

Based on the available data, the lead molecule *n*B-DNJ is a micromolar inhibitor of CGT, while DNJ, which lacks an alkyl group at the nitrogen, is not an inhibitor, demonstrating that an alkyl group at the nitrogen is crucial for inhibition. However, variations of the *N*-alkyl groups are seemingly possible. A longer alkyl chain, such as a nonyl group, is especially favorable. Since both the glucose- and galactose-derived iminosugars *n*B-DNJ and *n*B-DGJ are active compounds, the stereochemistry at position 4 is deemed not to be important for activity. *N*-Butyl-α-homo-nojirimycin and other C1 substituted analogues are inactive, indicating that the C1 position needs to be left unsubstituted. Methylether formation at *O*-3 is detrimental, suggesting that a free hydroxyl group at that position is necessary for enzyme inhibition. Removal or acetylation of the C6 hydroxyl group results in a large decrease in activity, therefore an intact hydroxyl group needs to remain at that position when new analogues are designed. A brief summary of the known SAR is shown in **Figure 4**.

Figure 4. Summary of SAR information



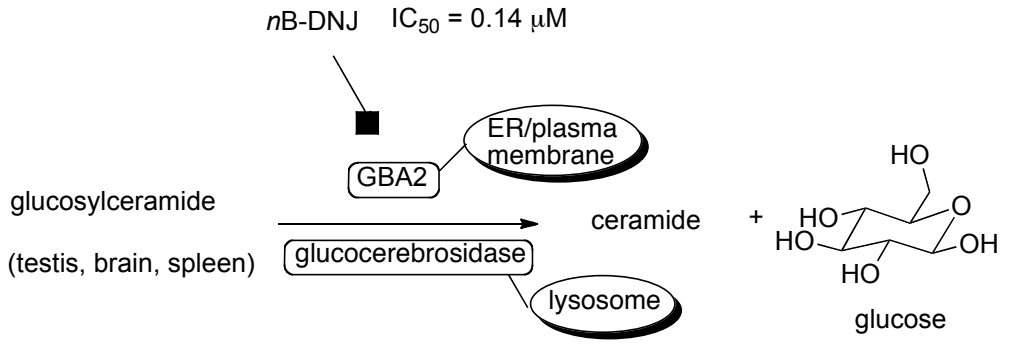
1.1.2 GBA2 as target of *n*B-DNJ

As discussed in the previous section, the sterility induced by *n*B-DNJ is species specific, which suggests that there may be other targets of *n*B-DNJ than CGT. In a more recent study, it was proposed by Walden and coworkers that *n*B-DNJ induces infertility in C57BL/6 mice through interaction with a different target than CGT.[26] The derailed acrosome formation was linked to an enzyme known as β-glucosidase 2 (GBA2).

This enzyme was first identified and cloned as microsomal β-glucosidase, or bile acid β-glucosidase.[27,28] It had long been known that *GlcCer* is primarily hydrolyzed into glucose and ceramide by the lysosomal enzyme glucocerebrosidase (GBA1). A non-lysosomal glucosylceramidase had also been defined but had never been characterized, until it was recently discovered that the non-lysosomal glucosylceramidase was identical to the previously mentioned GBA2, which governs the *GlcCer* catabolic pathway in extralysosomal locations,[29] as depicted in **Figure 5**. The lack of non-lysosomal glucosylceramidase activity leads to the accumulation of *GlcCer*. Indeed, genetically modified ($GBA2^{-/-}$) male C57BL/6 mice have increased levels of *GlcCer* in their testis, liver and brain. More importantly, GBA2-deficient male mice produced round-headed spermatozoa with abnormal acrosomes along with reduced fertility similar to those treated with *n*B-DNJ.[30] This implied that, perhaps, an elevation of *GlcCer* in the testis would have a disrupting effect on spermatogenesis. In addition to that, the glucosylceramidase activity of GBA2 was proposed to be susceptible to inhibition by *N*-alkylated iminosugars,

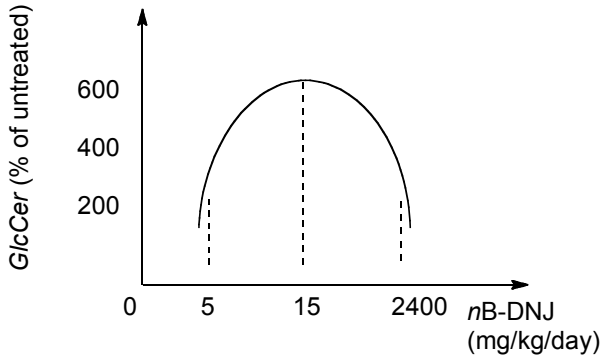
including *n*B-DNJ.[31] Taken together, the available information suggested that the elevation of *GlcCer* caused by the inhibition of GBA2, could be the first step of the cascade through which *n*B-DNJ and other iminosugars would cause C57BL/6 mice to produce impaired spermatozoa.

Figure 5. Inhibition of GBA2 by *n*B-DNJ



In order to investigate the biochemical basis of this notion, Walden and coworkers studied the variation of *GlcCer* levels in C57BL/6 mice caused by administration of *n*B-DNJ.[26] Interestingly, a biphasic relationship between oral dose of *n*B-DNJ and the testicular level of *GlcCer* was observed (**Figure 6**).

Figure 6. Relationship between dose of iminosugar and testicular *GlcCer* level



* This figure depicts a simplified biphasic relationship – see text for reference of experimental data

As shown in **Figure 6**, at lower doses (up to 15 mg/kg/day), *GlcCer* levels correlated with drug dose, reaching a maximum of six times control value at 15 mg/kg/day. At high doses (150-2400 mg/kg/day), the correlation was negative. At the highest dose used, the *GlcCer* level became similar to that of untreated animals.

This paradoxical relationship was explained by the difference in sensitivity of CGT and GBA2 toward inhibition by *n*B-DNJ. *In vitro* data showed that GBA2 was more sensitive than CGT toward inhibition by the iminosugar (IC_{50} values = 0.14 μM, 22.9 μM, respectively). Therefore it was believed that, *in vivo*, drug dosage determined which enzyme(s) was/were inhibited. At lower doses (up to 15 mg/kg/day), serum level of *n*B-DNJ (~ 0.5 μM) exceeded the *in vitro* IC_{50} toward GBA2 (0.14 μM), but remained well below the corresponding IC_{50} toward CGT; hence, *n*B-DNJ primarily inhibited GBA2 and rendered an increased testicular *GlcCer* level. At higher doses (150 - 2400 mg/kg/day, serum levels 1.7 - 21.5 μM), activities of both CGT and GBA2 were reduced, so that less *GlcCer* was produced, resulting in an overall decrease in *GlcCer* levels.

Even after the paradoxical relationship between the dose of *n*B-DNJ and *GlcCer* levels was explained, Walden's hypothesis still remains somewhat questionable. In his hypothesis, an elevated level of testicular *GlcCer* triggers the cascade that induces impaired spermatogenesis. If this hypothesis stands, one would expect to observe an increased level of *GlcCer* at any dose that induces infertility. However, the experimental data showed no increase of *GlcCer* when higher doses of the iminosugar were administrated, while higher

doses of *n*B-DNJ can certainly induce infertility (the minimum dose required is 15 mg/kg/day). To further support his theory, Walden proposed a model in which the higher amount of *GlcCer* that hampers spermatogenesis is localized in a particular (sub)cellular site. Therefore, at higher iminosugar doses, although the overall testicular *GlcCer* level is reduced, the amount of *GlcCer* in this particular location can still be high enough to trigger the cascade. Knowledge about the (sub)cellular location of GBA2 will support this model, yet the current data on the location of this enzyme are not in agreement with the theory.[29-31]

Moreover, Walden's hypothesis does not explain the species dependency of *n*B-DNJ's contraceptive effect. *GlcCer* levels were measured in ten inbred strains of mice other than C57BL/6. After treating males of these strains with low dose *n*B-DNJ, *GlcCer* levels were found to increase in all strains, to as high as the drug-treated C57BL/6 mice. Yet, males of these strains didn't show the reproductive phenotype, suggesting a possibility that other inbred strains may express allelic variants of the downstream enzymes/proteins that are less susceptible to *GlcCer* accumulation.[32] The fact that elevated levels of *GlcCer* failed to induce infertility in these males could also suggest that accumulation of *GlcCer* in testis is not responsible for impairment of spermatogenesis.

1.1.3 Summary of *n*B-DNJ's male contraceptive effect

In summary, the male contraceptive effect of *n*B-DNJ and other iminosugars on C57BL/6 mice is likely to be induced by inhibiting CGT and/or GBA2. Yet to date, no adequate SAR information is available for researchers to

design and develop an iminosugar-based male contraceptive agent. Also, the mechanism causing *n*B-DNJ's species specificity remains obscure. In order to clarify the basis of the species dependency, the potential unknown targets of the iminosugar remains to be investigated.

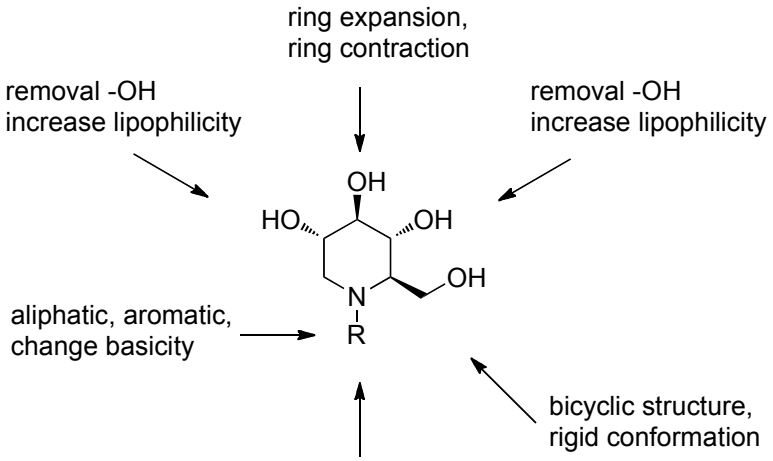
1.2 Objectives

Our goal is to synthesize *n*B-DNJ analogues targeting CGT and/or GBA2, and generate SAR data. The SAR information will be utilized to prepare agents with higher potency and improved pharmacokinetic properties. In addition, we propose to characterize and identify potential novel target proteins of *n*B-DNJ.

1.2.1 Structural modification strategies

The research described in this subsection describes the strategies that we applied to modify the iminosugar molecule, and the synthesis and evaluation of these analogues. The approaches that we used to modify the structure of the iminosugar included new *N-substituents*, changing the *molecular geometry*, *deoxygenation*, stereoisomers of the *-OH groups*, ring contraction and ring expansion, as shown in **Figure 7**.

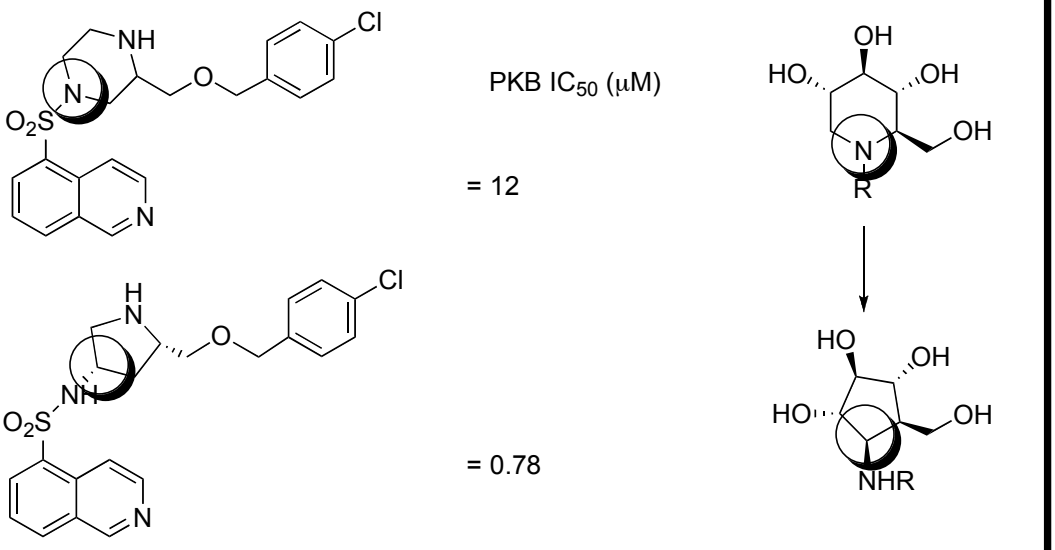
Figure 7. Medicinal chemistry approaches



Among these approaches, changing *N-substituents* has been frequently practiced because *C-N* bonds can be made easily. In order to explore the relationship between conformational flexibility and activity, we proposed to append relatively bulky substituents onto the nitrogen in order to restrict conformational flexibility.[33,34] The effect of reduced basicity on activity was to be evaluated by appending aromatic groups onto the nitrogen atom. The removal of one or more hydroxyl groups, that is (are) not relevant to activity, was expected to preclude phase II-type metabolism and therefore improve the half-life of the compounds. Changing molecular geometry, as depicted in **Figure 8**,[35,36] was to move the nitrogen atom toward an exo-cyclic position, increasing the distance between the nitrogen and the hydroxyl groups while retaining the composition of atoms and at the same time increasing basicity of the nitrogen. Ring

contraction/expansion analogues were prepared by other researchers in the Georg group, and not included in this section.

Figure 8. Molecular geometry change in drug design

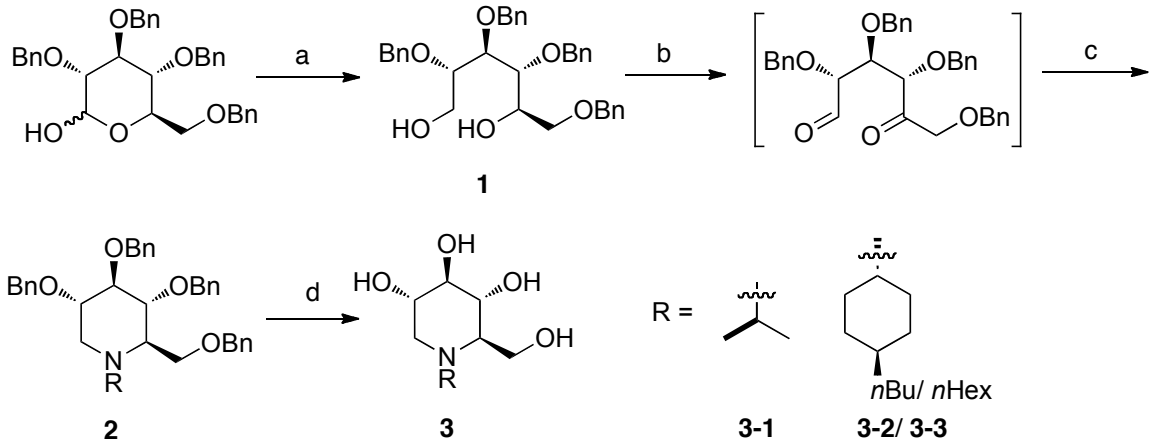


* This figure shows an example of a molecular geometry change in medicinal chemistry, in which the kinase inhibitory activity of the pyrrolidine analogue was enhanced by ~ 15-fold compared to the piperazine compound - see text for reference.

1.3 Synthesis of analogues with modified *N-substitution*

The first modification was to prepare analogues with sterically demanding lipophilic groups as *N*-substitutes, and to investigate the effect on the enzyme inhibitory activity of these iminosugars. The desired analogues were obtained in four steps[37] as outlined in **Scheme 1**.

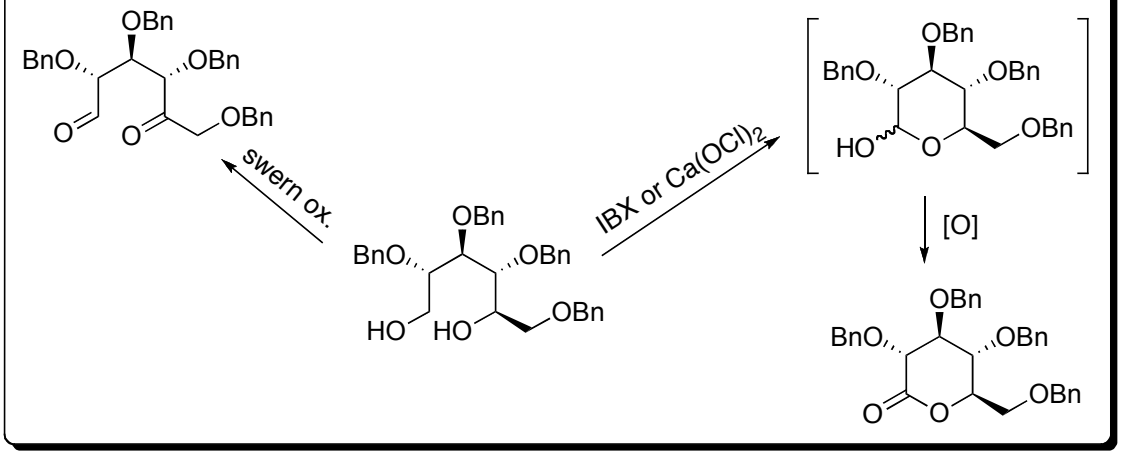
Scheme 1



a) LAH, THF, 0 °C to rt, overnight, *95%*; b) oxalyl chloride, NEt_3, DMSO, CH_2Cl_2, -78 °C; c) isopropylamine/**7**, $NaBH_3CN$, MeOH, 3Å MS *36% - 65%*; d) H_2, $PdCl_2$, *15% - 78%*

The diol **1** obtained from reduction of benzyl-protected D-glucose was subjected to a Swern oxidation to yield a chemically labile ketoaldehyde. The very mild nature of the oxidation was the key to the success of this transformation. Under Swern conditions, both the primary and secondary alcohol groups in diol **1** were oxidized, while with other oxidants (IBX, $Ca(OCl)_2$) the primary alcohol was oxidized to the aldehyde first, which reacted with the secondary alcohol to form a hemi-acetal. Subsequently, the hemi-acetal was oxidized to an undesired lactone as the main product, as shown in **Figure 9**.

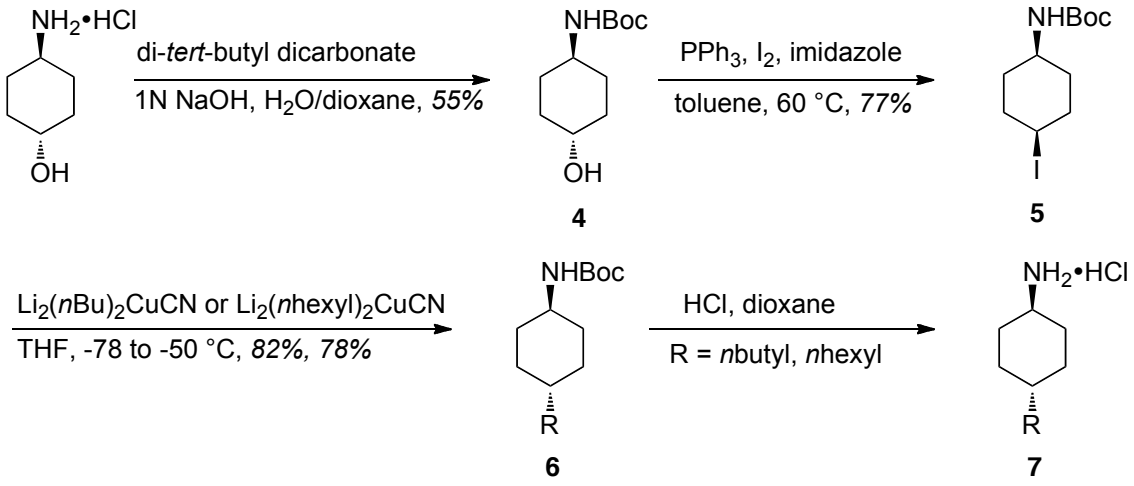
Figure 9. Swern oxidation vs. other oxidants



The ketoaldehyde intermediate was taken to the next step without isolation and subjected to a double reductive alkylation reaction with sodium cyanoborohydride to yield the *O*-benzyl-protected product **2**. In this transformation, the primary amine first forms an imine with the aldehyde; the imine is then reduced to give a secondary amine. The newly formed secondary amine then reacts intramoleculary with the ketone moiety to form a cyclic imine, which is subsequently reduced stereoselectively to yield the corresponding tertiary amine. The reagent sodium triacetoxyborohydride, which is also commonly used for reductive alkylations, was tried but produced an undesired dual-alkylated product and ring-closing was not achieved. To introduce steric bulk to the nitrogen atom, isopropylamine, *trans-n*butylcyclohexanamine and trans-*n*hexylcyclohexanamine were used in the double reductive alkylation reaction. The protecting groups were removed via hydrogenolysis, and yielded the desired iminosugars **3.1**, **3.2** and **3.3**.

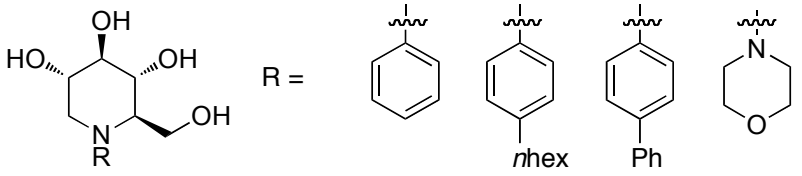
The two non-commercially available amines that were employed in the double reductive alkylation were prepared as shown in **Scheme 2**. The amino group of the starting material, *trans*-4-aminocyclohexynol hydrochloride, was liberated and Boc-protected using a literature method.[38] The hydroxyl group in **4** was subsequently replaced, yielding the iodo derivative **5**. Addition of cuprate reagents to **5** furnished 4-alkylated product **6**.[39] The Boc protecting group was then removed under acidic conditions and the HCl salts of the resulting amines were directly taken to the double reductive alkylation.

Scheme 2



Electron-withdrawing *N*-aryl substitutes (**Figure 10**) were also introduced, utilizing the same methodology depicted in **Scheme 1**. *N*-aryl groups were expected to reduce basicity of the nitrogen atom in the iminosugars and potentially to interact with π moieties in the enzyme active site.

Figure 10. *N*-aryl and *N*-morpholino analogues

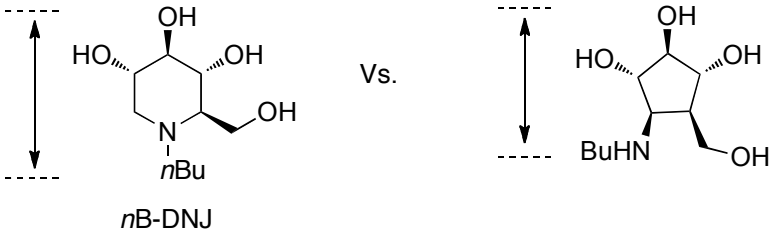


A *N*-morpholine group, which is a common building block in synthesis of therapeutics, was appended to the parent molecule in order to further probe the enzyme active site. The *N*-morpholine group could provide additional H-bond interactions with the enzyme.

1.4 Synthesis of analogues with *Molecular Geometry Change*

Hydroxylated carbocyclic amines, possessing an exocyclic nitrogen atom, resemble *n*B-DNJ closely, with respect to atomic composition and stereochemical arrangement of hydroxyl groups, as illustrated in **Figure 11**.

Figure 11. Comparison between *n*B-DNJ and hydroxylated carbocyclic amine

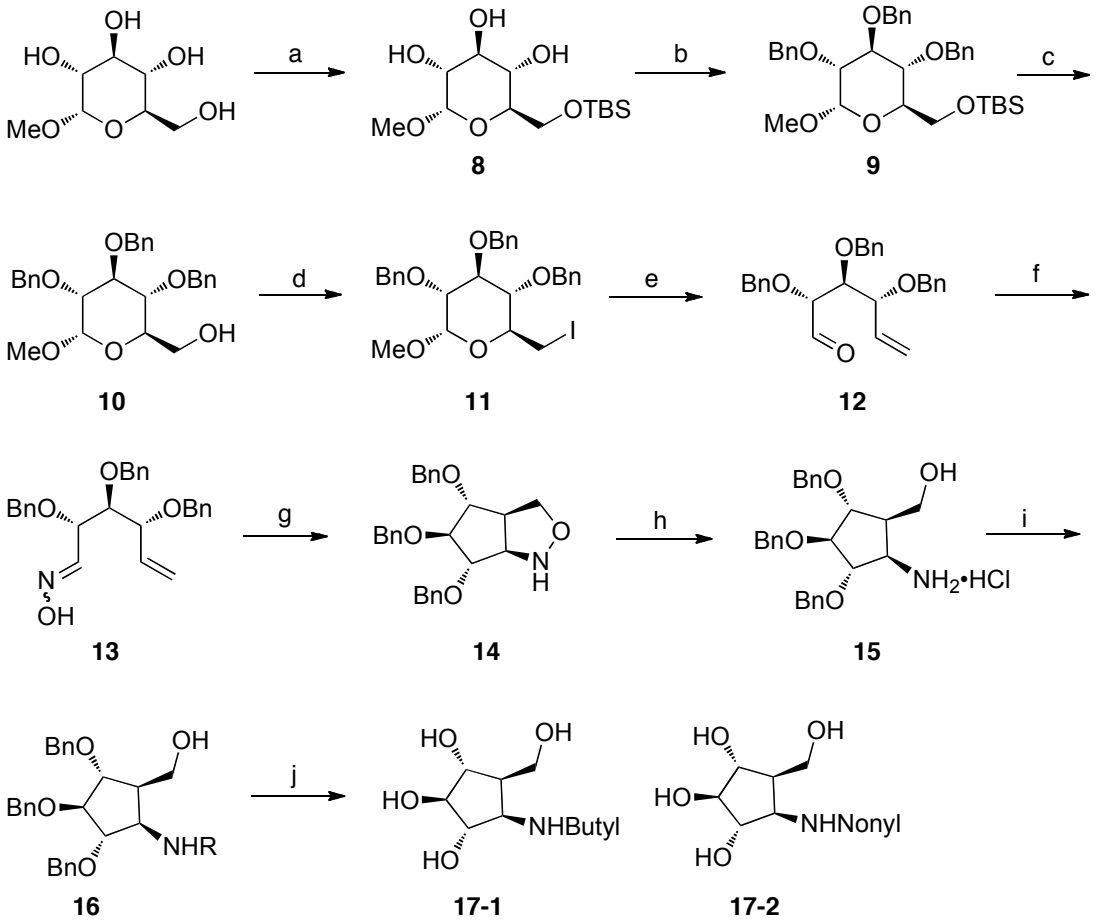


*n*B-DNJ

We proposed that the hydroxylated aminocyclopentanes could mimic *n*B-DNJ in its inhibitory activity based on structural similarities but would display increased basicity and less steric hindrance at the nitrogen atom. The four hydroxyl groups of the proposed aminocyclopentane share the stereochemical

configurations with *n*B-DNJ and are oriented in space similarly to those of *n*B-DNJ. Although the hydroxylated aminocyclopentane resembles *n*B-DNJ, the difference in geometry between these two structures is significant due to the alteration of the ring size. Therefore, hydroxylated aminocyclopentanes were anticipated to occupy the enzyme active center in a slightly different manner, and to possess altered inhibitory activities compared to *n*B-DNJ. The synthetic route leading to the hydroxylated cyclopentylamines is shown in **Scheme 3**.

Scheme 3

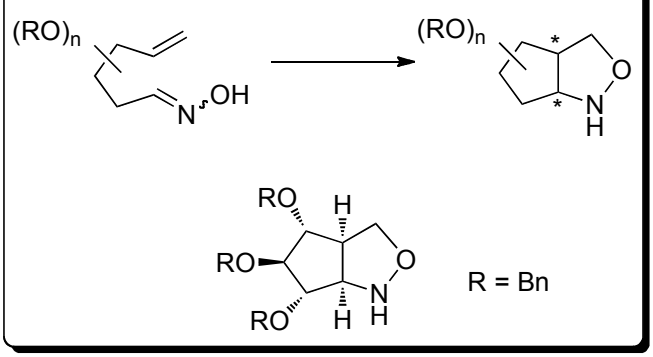


a) TBS-Cl, imidazole, DMF, 0 °C to rt, *90%*; b) BnBr, NaH, DMF, 0 °C to rt, *85%*; c) TsOH, MeCN/ H_2O, *65%*; d) PPh_3, imidazole, I_2, THF, reflux, *97%;* e) Zn, THF/H_2O, sonication, *94%*; f) NH_2OH•HCl, $NaHCO_3$, MeOH, reflux, 20h, *85%*; g) Toluene, reflux, *70%*; h) 1. Zn, HOAc/H_2O, *87%*, 2. HCl in ether; i) Butyraldehyde/nonylaldehyde, $NaB(OAc)_3H$, DCE, ~*70%*; j) Pd/C, $HCOONH_4$, MeOH, reflux, *42% or* Li/NH_3 -78 °C, *52%*

Our synthesis started with a selective protection of the primary alcohol of methyl-alpha-D-glucopyranoside as a *tert*-butyldimethylsilyl (TBS) ether.[40] The remaining free hydroxyl groups of **8** were protected as benzyl ethers to generate **9**. The TBS group was removed under acidic conditions to unmask the primary hydroxyl group of **10**, which was subsequently converted to iodo compound **11**. Aldehyde **12** was obtained via a reductive ring opening of **11**, which is known as the Vasella fragmentation reaction.[41] Initially a reported method was applied to achieve the fragmentation, which utilized Vitamin B_{12} as a catalyst in the zinc-mediated reaction.[42] In efforts to lower the cost, we discovered that a comparable yield could be obtained by performing the reaction in a THF/H_2O medium under ultrasonic conditions, without the need of a catalyst. Treatment of aldehyde **12** with hydroxylamine hydrochloride in warm methanol in the presence of sodium bicarbonate furnished oxime **13**, as a mixture of *E* and *Z* isomers.

An intramolecular oxime-olefin cycloaddition (IOOC) was used as the key step in our synthesis toward the stereochemically defined hydroxylated amino-cyclopentanes.[43,44] In this key transformation, two new stereogenic centers were created at the ring fusion, as shown in **Figure 12**. Literature data had shown that, when the glucose-derived oxime olefin was subjected to IOOC, the two hydrogen atoms at the ring junctions would be *cis* and reside on the *alpha*-face exclusively. Thus, this key reaction ensured the stereochemical outcome for the target molecule.

Figure 12. Stereoselective ring fusion of oxime **13**



The 1,3-dipolar cycloaddtion of oxime **13** in refluxing toluene for 15 hours led to *cis*-fused isoxazolidine **14**. The *N*-*O* bond was cleaved with activated zinc under acidic aqueous conditions to furnish amine **15**.[45] The reductive alkylation reaction of the purified amine **15** with aldehyde gave a mixture of mono- and di-alkylated products, which were difficult to separate. As a consequence, the isolated yield of the mono-alkylated amine was relatively poor. In an effort to improve the yield and simplify the handling of amine **15**, the HCl salt was prepared. The semi-crude residue from the organic extraction of **15** was dissolved in a solution of hydrochloric acid in ether. The salt precipitated and was then filtered, rinsed with ether and dried under reduced pressure to afford the HCl salt of amine **15**. When the salt (pre-dissolved in the presence of triethylamine) was taken to the reductive alkylation step, to our surprise the reaction produced monoalkylated products exclusively, providing an improvement in yields (*49→73%* and *41→70%*, respectively). Following that, the protecting groups of the *N*-butyl carbocyclic amine were removed under hydrogenolysis conditions to yield **17-1**, while Birch conditions[46,47] were needed to obtain the *N*-nonyl derivative **17-2**.

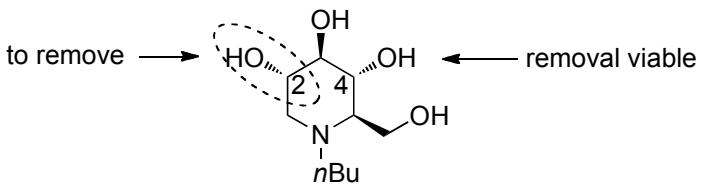
1.5 Synthesis of analogues with *modified -OH groups*

Polar drugs, such as *n*B-DNJ, can have relatively poor pharmacokinetic parameters, which can potentially jeopardize their effectiveness. In humans, the half-life of the iminosugar was observed to be 6.5 hours after the first dose of 100 mg, with a maximum serum concentration of only 0.86 μg/mL.[48] The relatively short half-life of *n*B-DNJ is due to rapid renal clearance, which is most likely a result of the hydrophilicity of the compound and the presence of several hydroxyl groups that can be subjected to phase II-type metabolism. It was expected that the half-life of the analogues could be incresed by removing one or two hydroxyl groups deemed to be unnecessary, while retaining activity.

1.5.1 2,4-Dideoxygenated analogue of DNJ

Based on currently available information, little is known about the role that the 2-OH group plays in iminosugars. In order to address this issue, the 2-deoxy-DNJ analogue (**Figure 13**), needed to prepared and assayed.

Figure 13. 2-Deoxy-DNJ analogue



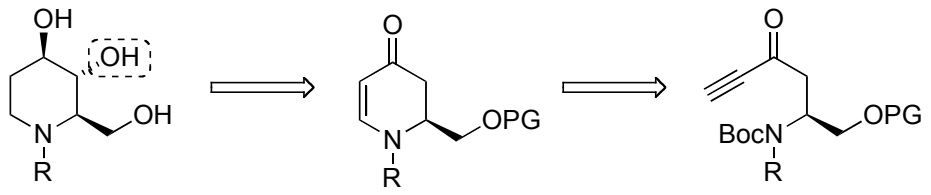
***n*B-DNJ**

As discussed previously, 4-*epi*-*n*B-DNJ (*n*B-DGJ) is an active inhibitor of CGT, suggesting that the 4-OH group perhaps contributes less to the inhibitory effect of the iminosugars than other hydroxyl groups, therefore the removal of the

4-OH may have little impact on the effectiveness of the corresponding analogue. Thus, the 2,4-dideoxy DNJ analogue, in which both hydroxyl groups are removed, would also be a good candidate for biological evaluation, with regards to testing the importance of the 2-OH group as well as the 4-OH group.

We envisioned that both the 2-deoxy- and 2,4-dideoxy-DNJ analogues could be derived from a common enaminone intermediate, as shown in **Figure 14**. The 4-OH was envisioned being installed via α-hydroxylation at the position that is α to the carbonyl group of the enaminone intermediate. A subsequent reduction was to furnish the 3-hydroxy functionality in the target molecule.

Figure 14. The key methodology employed to prepare 2-deoxy-DNJ analogues

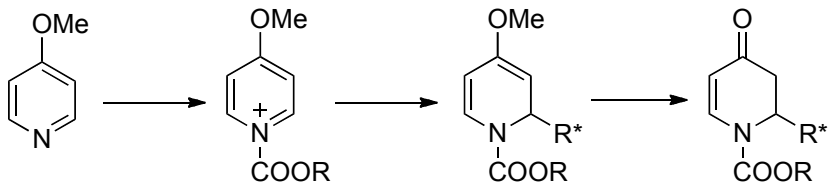


2-deoxy-, and 2,4-dideoxy analogue

Regarding the preparation of the enaminone intermediate, the Georg group has developed a practical method to generate such useful synthons.[49]

Prior to this discovery, the techniques utilized to prepare enaminones primarily originated from a method developed by the Comins group.[50,51] This method employs acylpyridinium salts prepared *in situ* from 4-methoxy-pyridines as starting material, as depicted in **Figure 15**.

Figure 15. Comins enaminone synthesis

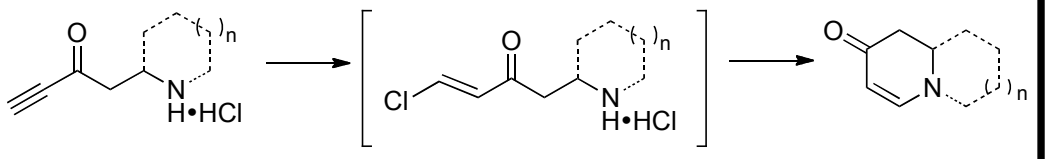


R = Ph, etc; R*= aryl, alkyl, H

The pyridinium salts are treated with Grignard reagents or sodium borohydride, and the resultant enol ethers are hydrolyzed to afford the corresponding enaminones.

Although the above method had proven enormously effective, the Georg group methodology provided a more direct route toward the same desired synthon and also in optically active form. In this method, the amino group of an amino-ynone system which can be easily prepared from β-amino acids, adds into an ynone to afford the enaminone moiety rapidly under basic conditions, as shown in **Figure 16**. Mechanistic studies revealed that the ring closure took place via the addition of the amino group into a vinylogous halide species, followed by elimination of the halogen, rather than through a direct Michael addition into the ynone (6-*endo*-dig).

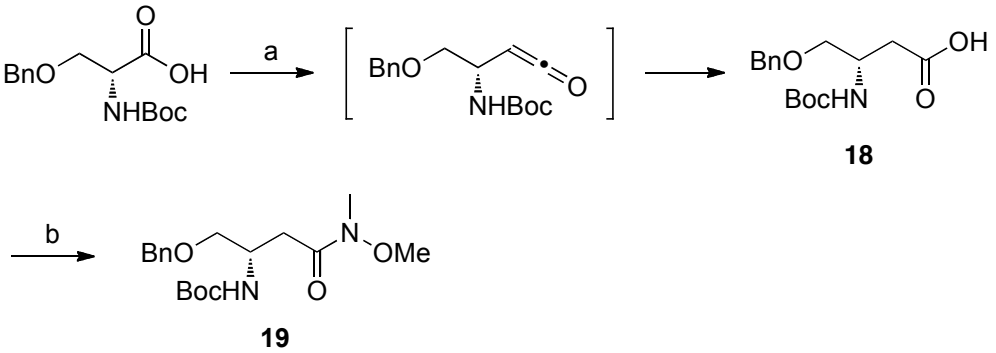
Figure 16. Enaminone synthesis developed in the Georg group



a) 4N HCl in dioxane; b) K_2CO_3, methanol, 15 min, *86-95%*

To synthesize our targeted 2-deoxy and 2,4-dideoxy-DNJ analogues, a β-amino acid derived from D-serine was envisioned as the starting material for the synthetic route. Although this β-amino acid derivative **18** was commercially available, it was rather expensive. Therefore, we chose to prepare **18** via an Arndt-Eistert reaction.[52,53]

Scheme 4



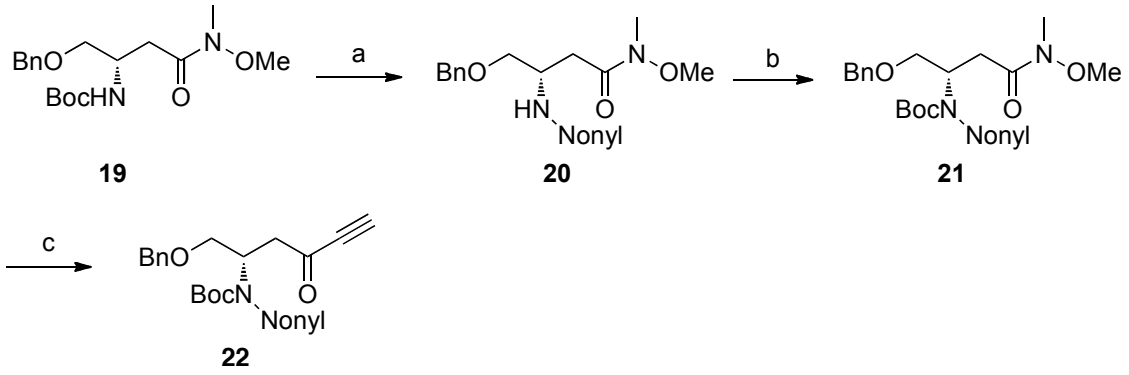
a) 1. $ClCO_2Et$, 2. CH_2N_2, 3. CF_3CO_2Ag, *86%*; b) EDCI, MeNHOMe•HCl, NMM, *92%*

The homologation reaction converted the *N,O*-protected α-D-serine to **18** in a two-step, one-flask fashion. The amino acid was reacted with ethyl chloroformate to furnish the corresponding carboxylic acid chloride, which was subsequently transformed into a diazo intermediate upon treatment with diazomethane. Safety was a concern, especially when the experiment was performed on large scale, since the highly explosive diazomethane was used. The diazo intermediate was purified via organic extraction. The semi-crude material was then treated with the silver salt of trifluroacetic acid, which catalyzed the Wolff rearrangement, to furnish a ketene species. The resultant ketene intermediate was hydrated to afford **18**. The β-amino acid was then converted to

Weinreb amide **19**,[54] as depicted in **Scheme 4**. The amide formation was rapid, clean and almost quantitative.

Efforts to directly introduce the alkyl chain onto the Boc-protected amine in compound **19** turned out to be unsuccessful under various conditions. The difficulty of the alkylation can be attributed to steric hindrance from both the Boc group and the alkyl halides. We then sought to accomplish the alkylation by utilizing a reductive alkylation reaction, although that lengthened the synthetic route by two additional steps. As a consequence, the Boc protecting group was removed under acidic conditions and the HCl salt of the crude product was taken to the reductive amination reaction, as shown in **Scheme 5**.

Scheme 5



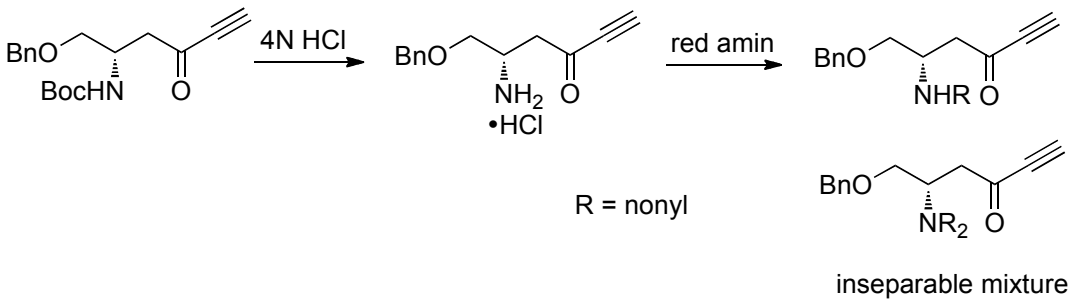
19 **20** **21**

a) 1. HCl, dioxane, 2. $NaBH(OAc)_3$, nonylaldehyde, DCE, rt, *85%*; b) Boc_2O, DMAP, MeCN, rt, *62%;* c) HCCMgBr, *70%*

The HCl salt was suspended in dichloroethane (DCE) and neutralized with one equivalent of triethylamine. The pH value of the reaction medium was then adjusted to be slightly acidic using acetic acid. Nonylaldehyde and sodium triacetoxyborohydride were added to the reaction in sequence. The reaction was was completed within two hours at room temperature. Dialkylation of the primary amine was initially detected, giving a modest yield (*45%)* of the desired mono-

alkylated product **20**. The dialkylation was suppressed by the using an equal molar amount or less of the aldehyde, which increased the yield to *85%*. The secondary amine was then re-protected with di-*tert*-butyl dicarbonate (Boc_2O) to provide compound **21** in moderate yield. The ynone **22** was easily obtained upon treatment of the Weinreb amide **21** with the commercially available Grignard reagent ethynylmagnesium bromide. We also attempted to switch the order of the above two steps, hence the ynone formation was carried out prior to the reductive alkylation, as shown in **Figure 17**. The incentive was to enhance the yield of the Boc re-protection step, in that the ynone molecule was significantly less sterically congested than the counterpart Weinreb amide. However, no clean separation of the mono- and dialkylated amines could be achieved, and this alternative route was therefore not pursued further.

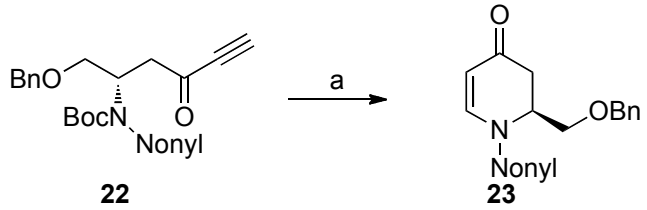
Figure 17. Ynone formation prior to reductive alkylation



To obtain the enaminone intermediate **23**, ynone **22** was dissolved in a solution of 4N hydrochloric acid in dioxane at room temperature. Within 20 minutes, all the starting material was consumed. After the dioxane was removed under reduced pressure, the remaining crude salt was dissolved in methanol at ambient temperature and potassium carbonate (K_2CO_3) was added. The ring

closure was completed instantly and the crude material was purified using flash chromatography, yielding the coveted cyclic enaminone **23** in excellent yield, as shown in **Scheme 6**.

Scheme 6

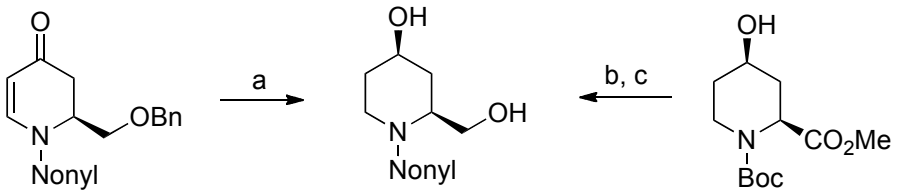


22 **23**

a) 1. HCl, dioxane, rt; 2. K_2CO_3, MeOH, *90%*

For the synthesis of the desired 2,4-dideoxy-DNJ analogue **24**, vinylogous amide **23** was subjected to a two-step, one-pot reduction.[55] An excess amount of sodium borohydride was used to reduce the carbonyl group and the double bond simultaneously, yielding the *cis*-hydroxyl derivative diastereoselectively. The excess reducing agent was then removed by organic extraction, and the structure of the intermediate was confirmed with ^{1}H NMR. The semi-crude material was subsequently hydrogenolyzed to cleave the benzyl ether protecting group, yielding the desire product **24**, as shown in **Scheme 7**. The NMR spectra of **24** were identical to those of a standard sample derived from a commercially available pipecolic acid derivative ((2S,4R)-*N*-Boc-4-hydroxypiperidine-2-carboxylic acid methyl ester, 98% *ee*). The specific rotation of diol **24** was comparable to that of the standard sample ($[\alpha]_D^{25}$ -22, *c* 0.75, MeOH, and $[\alpha]_D^{25}$ -21, *c* 0.97, MeOH, respectively), suggesting that no racemization took place during the cyclization step shown in **Scheme 6**. It was occasionally observed that the stereochemical integrity can be compromised when 6/6 and 6/5 bicyclic enaminones were generated using this chemistry.[49]

Scheme 7



23 **24** 98% *ee*

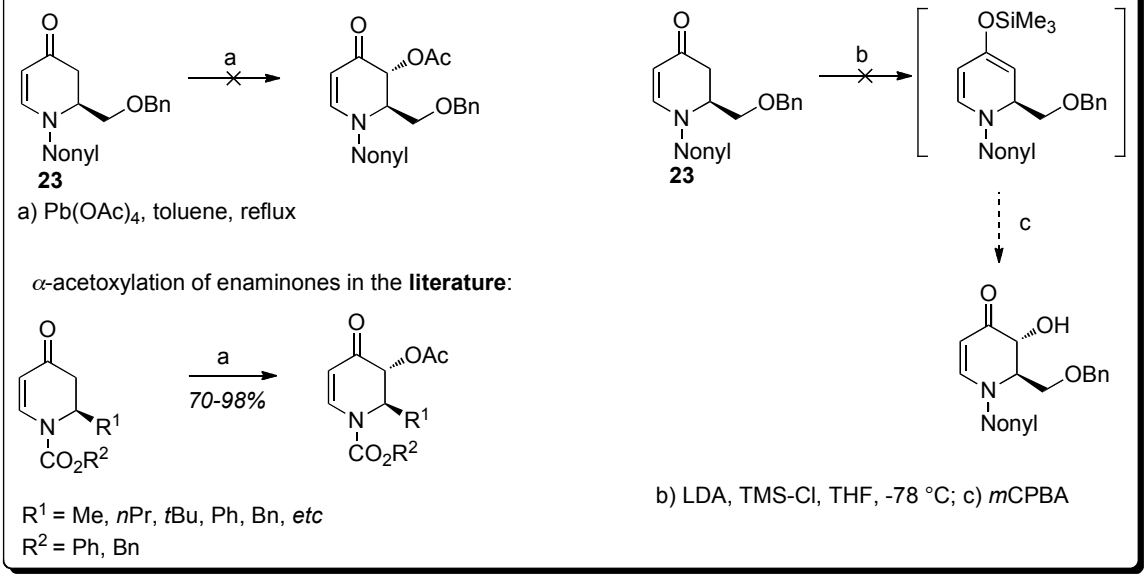
a) 1. $NaBH_4$, EtOH, rt; 2. H_2, Pd/C, *45%*; b) 1. 4M HCl, 2. nonylaldehyde, $NaB(OAc)_3H$, TEA, DCE, *96%*; c) DIBAL-H, toluene, *40%*

Targeting the 2-deoxy-DNJ analogue, we attempted α-hydroxylation on the vinylogous amide **23**, as depicted in **Figure 18**. A regio- and stereoselective α-acetoxylation, using lead (IV) tetra-acetate as the oxidant, was reported by Comins,[56] although the substrates used in this methodology differed from **23**, where electron-withdrawing groups were present on the nitrogen atoms. When enaminone **23** was subjected to the oxidation with $Pb(OAc)_4$ in refluxing toluene, only decomposition of the starting material was observed. Mechanistically, α-acetoxylation undergoes a key enolization step. The *N*-alkyl substituent in **23** presumably decreases the electrophilicity of the carbonyl group, making it less likely for the enolization to take place.

The difficulty in enolizing the carbonyl group was encountered again when we attempted to apply the Rubottom reaction[57,58] to **23**. No signs of the formation of the silyl-enol ether intermediate were observed (^{1}H NMR). Thus, the synthesis of the 2-deoxy-DNJ analogue was not pursued further.

To summarize this subsection, we completed the synthesis of the 2,4-dideoxy-DNJ analogue **24** in six steps, employing the amino-ynone cycloaddition methodology as the key step.

Figure 18. Attempts of α-hydroxylation of enaminone **23**

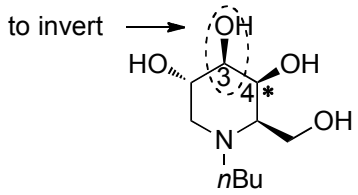


1.5.2 3-*epi*-DGJ analogue of DNJ

Only a few iminosugars containing hydroxyl group with inverted configuration have been prepared, and the properties of these "unnatural" iminosugars are not well understood. The stereochemistry of hydroxyl groups at carbons 2, 3 and 4 of *n*B-DNJ may all be systemically modified, and the effect of such modification on the ability to inhibit target enzymes can be measured. For example, *N*-butyldeoxygalactonojirimycin (*n*B-DGJ), which has an inverted hydroxyl group at C4, was tested against CGT and GBA2 and proved to be an active inhibitor of both enzymes (**Figure 19**). A comparison of the IC_{50} values of *n*B-DNJ and *n*B-DGJ indicated that inversion of the 4-OH stereochemistry had no significant influence on CGT inhibition. Yet, the outcome of inverting the

configuration of the C2 and/or C3 hydroxyl group(s) was unknown, even though such information is important to complete the SAR profile of the iminosugars.

Figure 19. *n*B-DGJ and 3-*epi*-DGJ analogue



***n*B-DGJ**

IC_{50} CGT = 30.0 μM
GBA2 = 5.3 μM

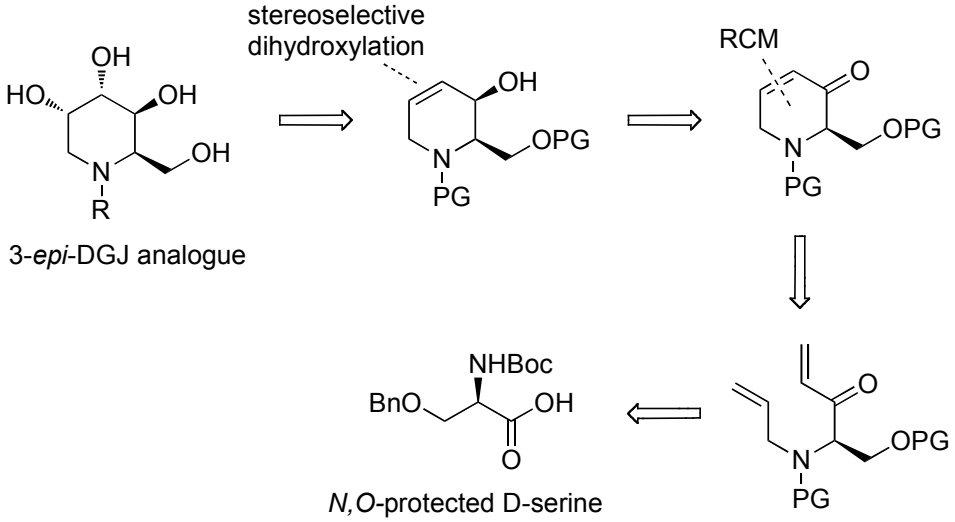
***n*B-DNJ**

IC_{50} CGT = 20.4 μM
GBA2 = 0.14 μM

In order to generate SAR data that can be examined, the inversion of the 2- and 3-OH groups should be conducted one at a time. Since the importance of the 2-OH group was unclear (seen in the previous section), we decided to prepare and study the 3-OH inverted analogue, leaving the 2-OH stereochemistry intact.

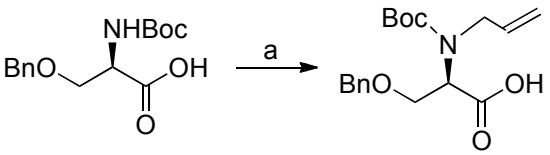
We envisioned that two of the α-hydroxyl groups of the 3-*epi*-DGJ analogue could be introduced in a stereoselective fashion (**Figure 20**), taking advantage of the directing effect of an endocyclic allylic alcohol. The allylic alcohol was envisaged to arise from a 1,2-reduction of an enone intermediate. The C-C double bond of the enone moiety was to be generated via a ring-closing metathesis reaction (RCM). The retrosynthetic product of the RCM was envisaged to come from the starting material *N,O*-protected D-serine, which was employed in the route leading to the 2,4-dideoxy-DNJ analogue in the previous section.

Figure 20. Retrosynthetic analysis of 3-*epi*-DGJ analogue

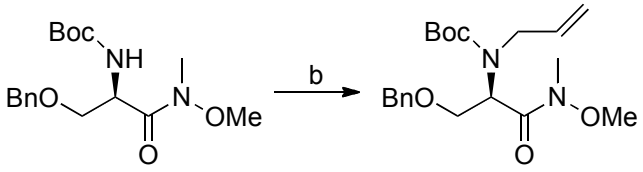


The installation of an allyl group onto the Boc-protected nitrogen of the amino acid turned out to be an obstacle (**Figure 21**). We first attempted to repeat a reported method,[59] in which *N,O*-protected serine was directly alkylated with allyl iodide in the presence of sodium hydride at low temperature for three days. Although this method was reported to give the product in high yield and purity, we found that additional purification was necessary before the *N*-allyl amino acid could be taken to the next step. Furthermore the isolated yield was not satisfying. We also tried to apply the alkylation to the corresponding Weinreb amide, yet the reaction was plagued by side reactions and yields were poor as well.

Figure 21. Attempts to install the *N*-allyl moiety



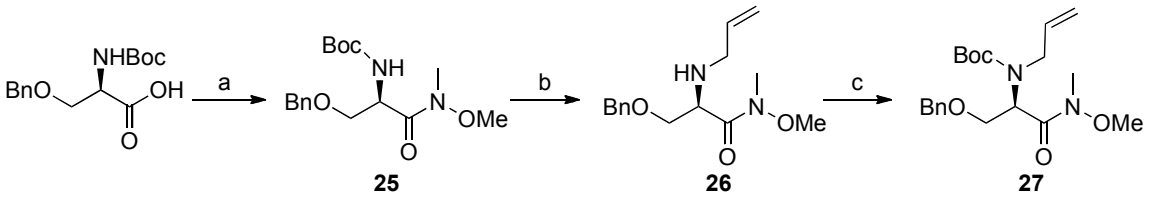
a) NaH, allyl iodide, 0 °C, THF, 72h, ~*30%*



b) NaH, allyl bromide, 0 °C, THF, *24%*

We then slightly modified the route so that we could obtain better yields, achieve a more convenient purification and reduce the risks of epimerization (**Scheme 8**). *N,O*-protected-D-serine was converted to Weinreb amide **25** using an EDCI-mediated coupling reaction. The Boc protecting group of **25** was then removed under acidic conditions. Without purification, the resultant HCl salt was dissolved in methanol and then Hünig's base (diisopropylethylamine, DIPEA). and allyl bromide were added in sequence.

Scheme 8



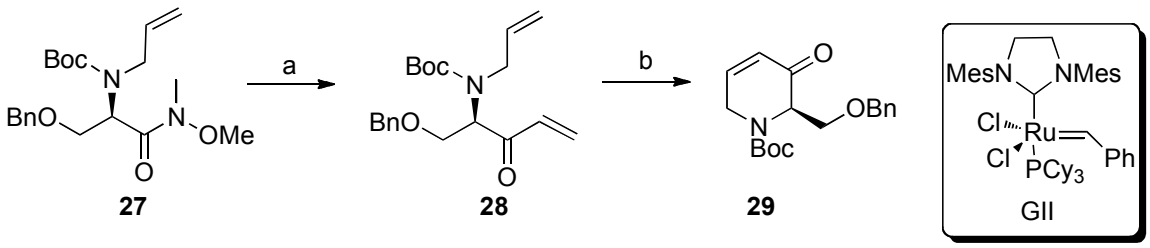
a) MeNH(OMe)•HCl, EDCI, NMM, 0 °C to rt, *90%*; b) 1. HCl/dioxane, 0 °C; 2. DIPEA, allyl bromide, MeOH 50 °C, *50%*; c) $(Boc)_2O$, EtOAc, rt, *90%*

The reaction was stirred at 50 °C for five hours, providing the *N*-allyl intermediate **26** in *50%* yield over two steps. Although the yield was only moderate, the purification turned out to be easy as the major side product, the

dialkylated product, was easy to remove. The allylamino group of **26** was then protected with di-*tert*-butyl dicarbonate (Boc_2O) in freshly distilled ethyl acetate (EtOAc) at room temperature to afford **27** in excellent yield.

Treatment of **27** with vinylmagnesium bromide at low temperature gave Weinreb ketone **28**, in excellent yield as shown in **Scheme 9**. The cyclic enone **29** was then obtained in *75%* by treatment of the diene **28** with the second-generation Grubbs catalyst (G-II).[60,61]

Scheme 9

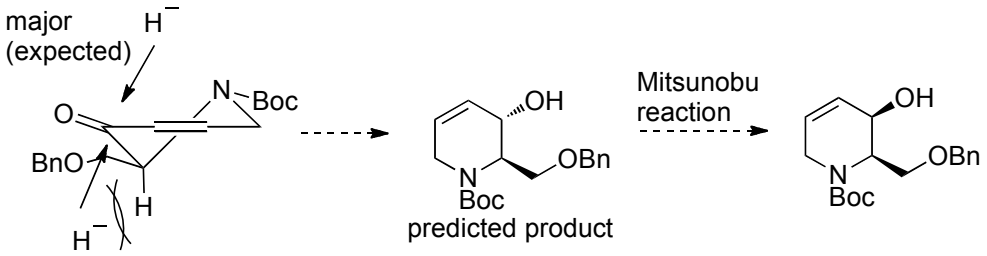


27 **28** **29**

a) vinylmagnesium bromide, THF, -78 to 0 °C, *90%*; b) G-II, DCM, reflux, *75%*

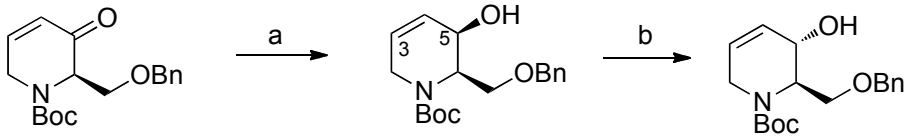
The subsequent step was the 1,2- reduction of the resultant enone **29**. We chose the Luche protocol,[62] to selectively reduce the conjugated ketone. With regards to the diastereoselectivity reduction of chiral ketones under Luche conditions, we expected that an axial hydride delivery would take place on enone **29**, which would generate an equatorial allylic hydroxyl group, as depicted in **Figure 22**. Since an axial hydroxyl group was needed for the following dihydroxylation, a Mitsunobu reaction was planned in advance, in order to invert the configuration of the -OH group.

Figure 22. Predicted stereochemical outcome of the Luche reduction



The 1,2-reduction of **29** was carried out with sodium borohydride in combination with cerium trichloride ($CeCl_3$) at low temperature, and was completed within 15 minutes, yielding a *cis*-alcohol, to our surprise, as the exclusive product, as shown in **Scheme 10**.

Scheme 10

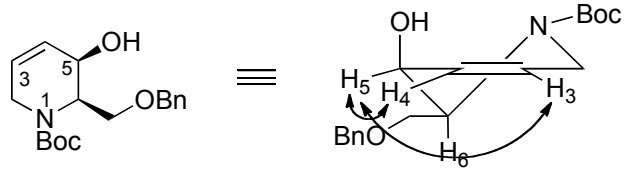


29 **30** 5-*epi*-**30**

a) $NaBH_4$, $CeCl_3$, MeOH, -78 °C to rt, 15 min, *92%*; b) 1. PPh_3, *p*$NO_2C_6H_4CO_2H$, DIAD, THF, 0 °C, *81%*, 2. K_2CO_3, MeOH, rt, *60%*

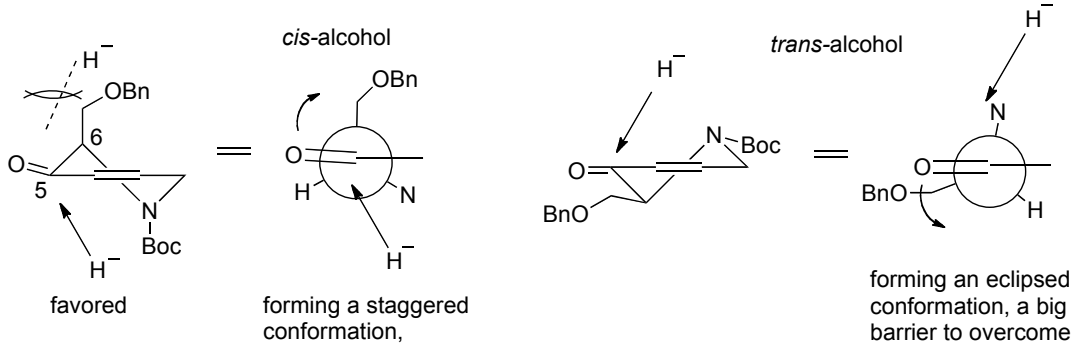
The stereochemical outcome was verified through ^1H, ^{13}C and 2-dimensional NMR experiments. The allylic proton H5 (δ = 4.53 ppm) appears further downfield compared to its counterpart (δ = 4.11 ppm) obtained from a Mitsunobu reaction. This observation indicates that H5 is located in the de-shielding cone of the C4-C5 bond, suggesting H5 orients as an equatorial proton.[63] A NOESY experiment further confirmed the configuration of H5, in that strong correlations were observed between H5 and H4, and even between H5 and H3 (**Figure 23**).

Figure 23. Results of NOESY experiment



The stereochemical outcome was clearly different from what was predicted. To explain the unexpected result, we propose that, prior to hydride delivery, the cyclic enone adopts a conformation in which the methylene benzylether side chain is in a pseudoaxial orientation, as depicted in **Figure 24**.[64,65] Thus, the steric hinderance rendered by the methylene benzylether side chain blocks the attack of the hydride from the β-face. As a result, delivery of the nucleophile from the α-face is favored, furnishing the *cis*-alcohol.

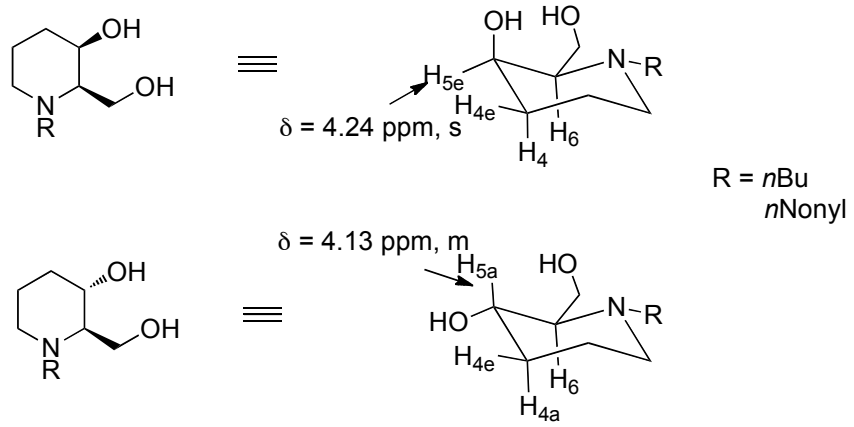
Figure 24. *Pseudo*axial delivery of hydride



In contrast to the favored *cis*-alcohol formation, the hypothetical *trans*-alcohol formation would have to overcome an energy barrier caused by a conformational change (from gauche to eclipse). Therefore, the formation of a *trans*-alcohol was not favored.

To further confirm the stereochemical outcome of the Luche reduction, the allylic alcohol **30** and the Mitsunobu reaction product were *N*-alkylated and reduced to diols as shown in **Figure 25**, and NMR spectra of these diastereomers were analyzed.

Figure 25. Further analysis of the stereochemical outcome

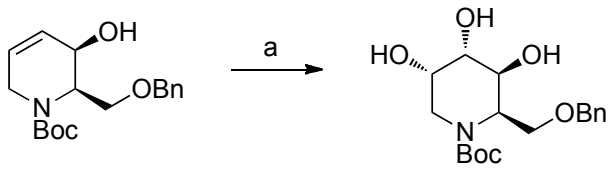


The stereochemistry was further confirmed via comparison of the vicinal coupling constant value of proton H5 (δ = 4.24 ppm) of the Luche reduction product to its counterpart (δ = 4.13 ppm) derived from the Mitsunobu reaction. The equatorial H5 of the diol directly derived from the Luche reduction appears as a singlet, suggesting H5 is not coupled to other hydrogens, which can be partially explained by the small dihedral angels between H5e and the neighboring hydrogen atoms. Conversely, in the product derived from the Mitsunobu reaction, these dihedral angels are increased due to the inversion of H5. As a result, the axial H5 appears as a multiplet in the spectrum as anticipated. We further deciphered the H5 multiplet utilizing the Hoye method,[66,67] and determined three coupling constant values for H5a: *J* = 4.0, 6.4 and 10.6 Hz.

The large coupling constants suggested the increased dihedral angels between H5 and neighboring hydrogens and therefore confirmed the relative positions of these coupled hydrogen nuclei.

The *cis*-allylic alcohol **30** was next subjected to dihydroxylation under Upjohn conditions, as shown in **Scheme 11**. The Upjohn dihydroxylation allows the *syn*-selective preparation of a diol from the alkene by using a catalytic amount of the toxic and volatile osmium tetroxide (OsO_4) and a stoichiometric amount of *N*-methylmorpholine *N*-oxide (NMO) as an oxidant, which can re-oxidize the Os(VI) species back to the Os(VIII) oxidation state.[68]

Scheme 11

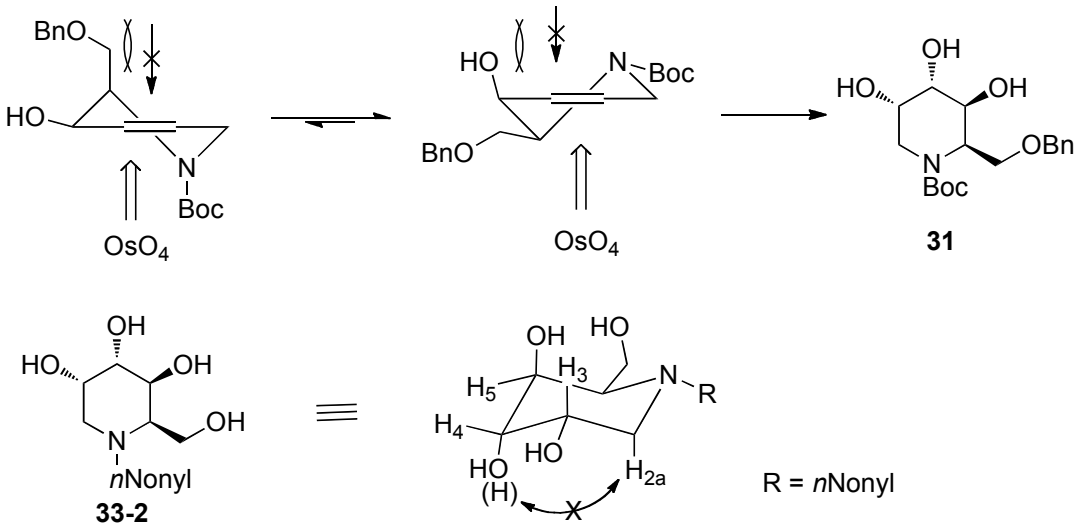


30 **31**

a) OsO_4, NMO, DCM/H_2O, 0 °C to rt, *78%*

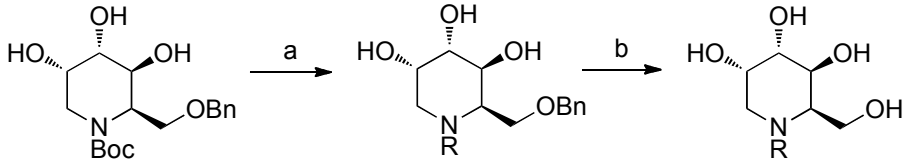
OsO_4 behaves as a large reagent, which attacks the C=C bond from the least hindered side, as shown in **Figure 26**. Thus, the dihydroxylation was highly diastereoselective (**30** → **31**), taking place *anti* to the 4-OH group.[65,69]

Figure 26. Diastereoselective dihydroxylation under Upjohn conditions



The stereochemistry of the newly introduced hydroxyl groups was also confirmed by a NOESY experiment on derivative **33-2**, where no correlation was observed between the axial H2a and H4, suggesting little through-space interaction was present between these two hydrogen atoms. H4 was then assigned as an equatorial proton, which is further away from H2a in space compared to a H4a would be. Thus, the 3- and 4-OH groups were confirmed to reside on the α face of the molecule.

Scheme 12



31 **32-1, 32-2** **33-1, 33-2**

R = *n*Bu, *n*Nonyl

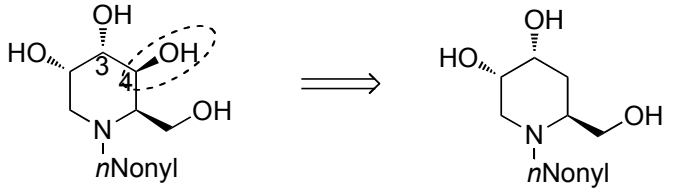
a) 1. HCOOH, rt, 2. $NaB(OAc)_3H$, butyraldehyde/nonylaldehyde, rt, *73%*, *71%*; b) Pd/C, $HCOONH_4$, MeOH, reflux, *66%*, *88%*, respectively

The Boc protecting group of triol **31** was removed under acidic conditions, and a following reductive alkylation reaction produced *N*-alkyl compounds **32-1** and **32-2**, as shown in **Scheme 12**. The desired 3-*epi*-DGJ analogues **33-1** and **33-2** were obtained after hydrogenolysis of the *O*-benzyl ethers, and purification by column chromatography.

1.5.3 3-*epi*-4-deoxy-DNJ analogue

We hypothesized in the previous section that the 4-OH group of *n*B-DGJ contributed little to its bioactivity. Removal of such hydrophilic hydroxyl group(s) could decrease the hydrophilicity of the resultant analogues and potentially reduce renal clearance caused by phase-II type metabolism, therefore providing an extended half-life. Based upon this hypothesis, we reasoned that the 3-*epi*-4-deoxy analogue (**Figure 27**) could display a better pharmacokinetic profile than the parent compound and also serve as a probe to further illustrate the effect of the sterochemical inversion of the 3-OH group.

Figure 27. 3-*epi*-4-deoxy-DNJ analogue



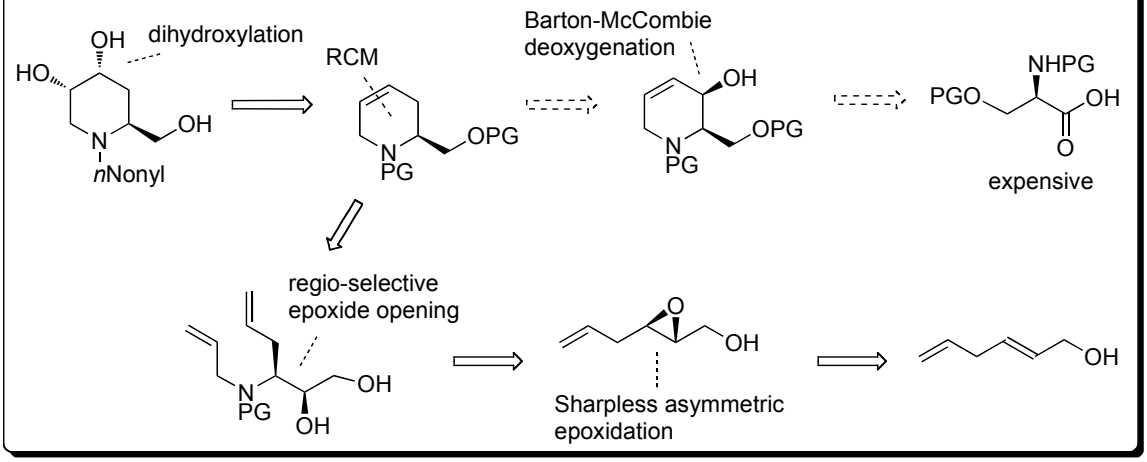
31-2 3-*epi*-4-deoxy-DNJ analogue

We envisaged that the *syn*-diol of the target compound could be introduced via dihydroxylation (**Figure 28**). The olefin intermediate could be obtained from a Barton-McCombie radical deoxygenation from an allylic alcohol

derived from D-serine. Although this route seemed feasible, as the allylic alcohol was successfully synthesized in the previous section, the cost of the starting material, D-serine, appeared to be a major drawback of this approach.

An alternative method was selected, employing a less costly starting material, as shown in **Figure 28**. Thus, the cyclic alkene was envisioned to come from a ring closing metathesis (RCM) of a diene. The diene could be derived from a regioselective epoxide ring opening with allylamine. The enantioenriched epoxide could be obtained from a Sharpless asymmetric epoxidation (SAE) of the resultant allylic alcohol, which could be prepared in large quantities from readily available reagents.

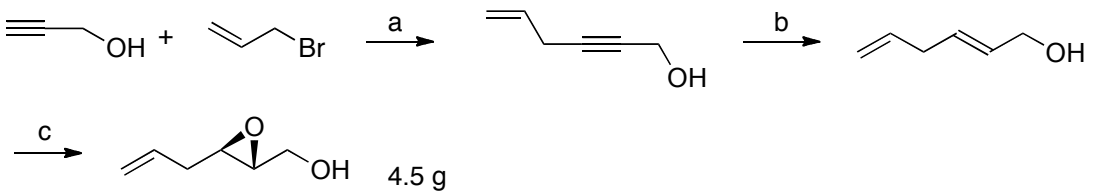
Figure 28. Retrosynthetic analysis of 3-*epi*-4-deoxy-DNJ analogue



The starting asymmetric oxirane was prepared on gram scale, following reported procedures[70,71] as shown in **Figure 29**. Propargyl alcohol was alkylated with allyl bromide in the presence of copper bromide (CuBr) at 70 ºC in a slightly basic aqueous solution, to yield hex-5-en-2-yn-1-ol. The product was then reduced with lithium aluminum hydride (LAH) to give the *trans*-allylic alcohol. The resultant allylic alcohol was subsequently subjected to a Sharpless

asymmetric epoxidation[72] (SAE) in the presence of diethyl-D-(-)-tartrate (D-(-)-DET), titanium tetraisoproxide (Ti(O*i*Pr)$_4$) and cumene hydroperoxide, to furnish (2*R*,3*R*)-epoxyhex-5-en-1-ol on gram scale. The reaction was carried out at low temperature and was kept free of moisture. The tartrate catalyst was prepared by mixing the titanium, tartrate and the hydroperoxide for over 30 minutes at -20 °C prior to the addition of the allylic alcohol, in order to achieve high enantioselectivity ([α]$^{22}_D$ 26.4, *c* 1.0, MeOH; lit. [α]$^{22}_D$ 23.2, *c* 10, MeOH).

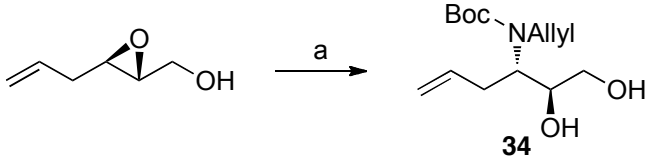
Figure 29. Preparation of the allylic epoxide as starting material



a) CuBr, H_2O, NaOH, pH = 8 to 9, 70 °C, *84%*; b) LAH, THF, 0 °C → rt → 45 °C → 0 °C, *37%;* c) cumene hydroperoxide, Ti(O*i*Pr)$_4$, 4Å MS, D-(-)-DET, -25 °C, *60%*

The enantioenriched oxirane was then subjected to a regioselective ring opening with allylamine,[73,74] followed by protection of the secondary amine with Boc_2O (**Scheme 13**) in order to avoid unwanted side reactions in the ring-closing metathesis step.

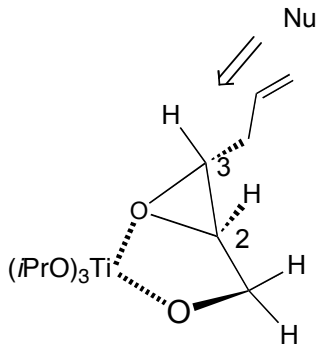
Scheme 13



a) 1. Ti(O*i*Pr)$_4$, allylamine, DCM, reflux, *44%*; 2. Boc_2O, $NaHCO_3$, sonication, MeOH, *76%*

The chelation-facilitated nucleophilic attack of the epoxide ring produced a mixture of regioisomers using Crotti's conditions,[75,76] which required a large access of reagents (15 equiv of $LiClO_4$ and 10 equiv of allylamine in acetonitrile). Due to the difficulties to separate the isomers, we resorted to the Sharpless conditions[77] which utilized smaller amounts of reagents (2 equiv of $Ti(O\textit{i}Pr)_4$, and 1 equiv of allylamine in DCM). To our delight, the Ti-mediated reaction provided the C3 openened aminodiol selectivity (**Figure 30**), although the yield was only moderate.

Figure 30. Ti-mediated epoxide opening



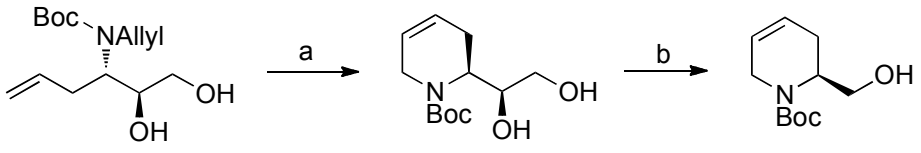
rigid, bidentate coordination to the metal center

The following Boc protection of the resultant secondary amine turned out to be not so straightforward. The reaction was extremely slow under regular conditions for Boc- protection. Fortunately, we found the reaction could be accelerated with ultrasonic treatment and the completion of the reaction was achieved after 6 days.

Treatment of diene **34** with the second-generation Grubbs' catalyst (GII) in DCM at room temperature gave the key intermediate **35**, as shown in **Scheme 14**. The oxidation of diol **35** with sodium periodate ($NaIO_4$), followed by reduction

of the resultant aldehyde with $NaBH_4$, afforded alcohol **36** in *60%* yield over two steps.

Scheme 14

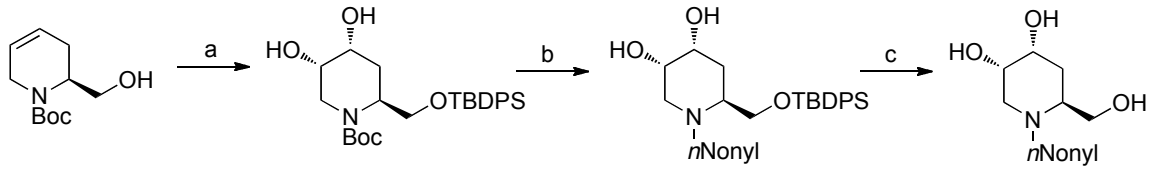


34 **35** **36**

a) Grubbs II, DCM, rt, 7*4%;* b) 1. $NaIO_4$, THF/H_2O; 2. $NaBH_4$, EtOH, *60%*

It turned out to be cumbersome to directly subject **36** to dihydroxylation and the following reductive alkylation, as it was difficult to isolate the aminotriol reaction product because of its polarity. In addition, the aminotriol was not reactive enough for the reductive alkylation. Thus, in order to solve these problems, the primary alcohol of compound **36** was first protected as a silyl ether with *t*-butyldiphenylsilyl chloride, and then subjected to Upjohn conditions to afford diol **37** as the major diastereomer (**Scheme 15**).[78] The hydrophobic silyl-protecting group enabled a more convenient isolation of the product. We also investigated the *t*-butyldimethylsilyl (TBS) silyl ether derivative of **36**, but that ether appeared to be resistant to dihydroxylation.

Scheme 15



36 **37** **38** **39**

a) 1.TBDPSCl, imidazole, DCM, rt; 2. OsO_4, NMO, DCM/H_2O, 0 °C to rt, *60%*; b) 1. HCOOH, 2. $NaB(OAc)_3H$, nonylaldehyde, DCE, *67%*; c) TBAF, THF, *96%*

The Boc protecting group of diol **37** was then removed under acidic conditions, and the *N*-nonyl group was introduced utilizing a reductive alkylation

reaction to furnish tertiary amine **38**. Finally, the silyl protecting group was removed by treatment with tetrabutylammonium fluoride (TBAF) to yield the desired product **39**. The polar aminotriol **39** was initially contaminated with tetrabutylammonium salts, which could not be removed by chromatography. The contaminants were removed by passing through a Dowex ion-exchange column using water as the eluent, to yield pure **39**.

1.6 Biological evaluation

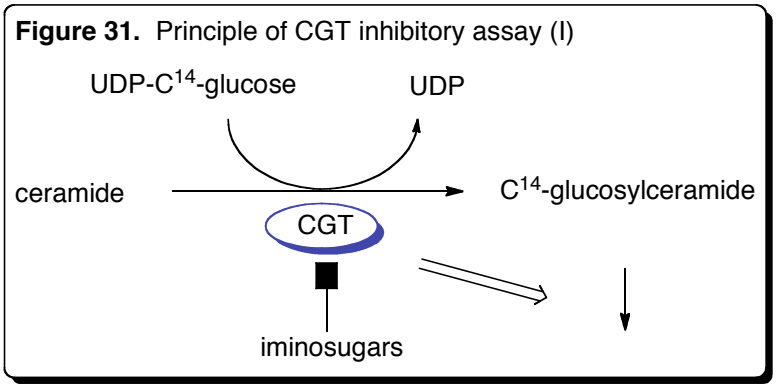
The biological evaluation of the iminosugars was conducted by our collaborators, Dr. Tash and his group, at the University of Kansas, Medical Center.

1.6.1 CGT inhibitory assay

The iminosugar analogues were first screened as CGT inhibitors.

1.6.1.1 Assay development

The published assay procedures were found unreliable and not reproducible.[15,24,25] Thus, new assays were designed and standardized, utilizing labeled reagents as reporters of the enzymatic reaction progress. The general scheme of the first assay applied is depicted in **Figure 31**.

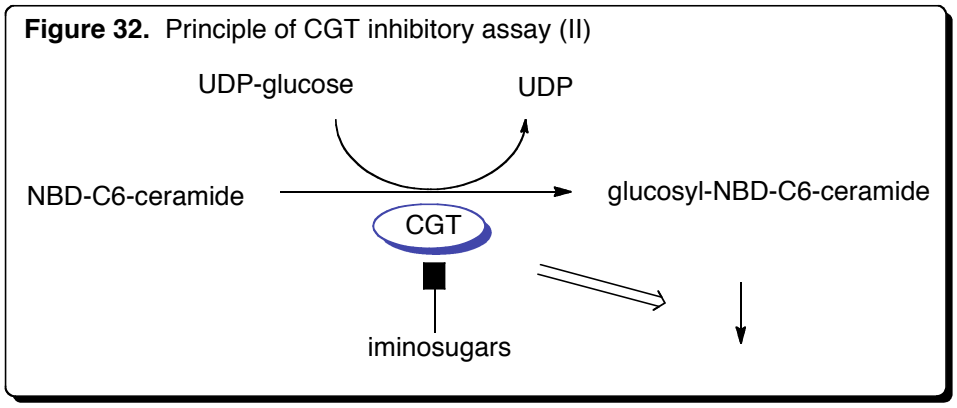


CGT catalyzes the glycosidation reaction of ceramide, yielding C^{14}-labelled glucosylceramide (C^{14}-*GlcCer*) as the product. C^{14}-*GlcCer* can be separated utilizing thin layer chromatography (TLC) and be quantified by autoradiography. Active iminosugars inhibit the enzymatic reaction, resulting in a decrease in the formation of C^{14}-*GlcCer*. Thus, the potency of the analogues correlates negatively with the intensity of signals detected.

Prior to addition of inhibitors, strong and consistent C^{14}-*GlcCer* signals were expected, serving as the positive control for the following screen. However, no readable signals were detected for C^{14}-*GlcCer* on the X-ray film. Numerous efforts were made to troubleshoot the problems, but no improvements could be made. Hence, a new assay was developed in order to evaluate the inhibitory activities of the synthesized iminosugars.

A practical *in vitro* assay was set up, using a ceramide derivative, NBD-C6-tagged ceramide ((6-((*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoyl)sphingosine)) as a fluorescent reporter of the enzymatic reaction. Using NBD-C6-tagged ceramide covalently linked with BSA (1:1), it

provides a method for detecting ceramide and the reaction product *GlcCer* after separation on silica gel thin layer plates (TLC).



As shown in **Figure 32**, active iminosugars inhibit the enzymatic reaction, inducing a decrease in product formation. Therefore, the potency of the analogues correlates negatively with the intensity of fluorescent signals.

Figure 33 shows the results when testis microsomes were incubated for 2-30 minutes with NBD-C6-ceramide. The starting substrate is indicated at the top of the TLC plate. The reaction product migrates more slowly and resides in the middle of the plate after development of the plate was completed. The NBD-tagged ceramide and *GlcCer* fluoresce green and can be easily distinguished from other non-specific fluorescent spots on the plate. The row of spots just above the *GlcCer* belongs to a fluorescent substance endogenous to the microsome preparation. A time-dependent increase in *GlcCer* formation is also indicated in **Figure 33**, which starts at 5 minutes and lasts until 20 minutes.

Figure 33. TLC separation of NBD-C6-ceramide and NBD-C6-*GlcCer* in control incubations with mice testicular microsomes

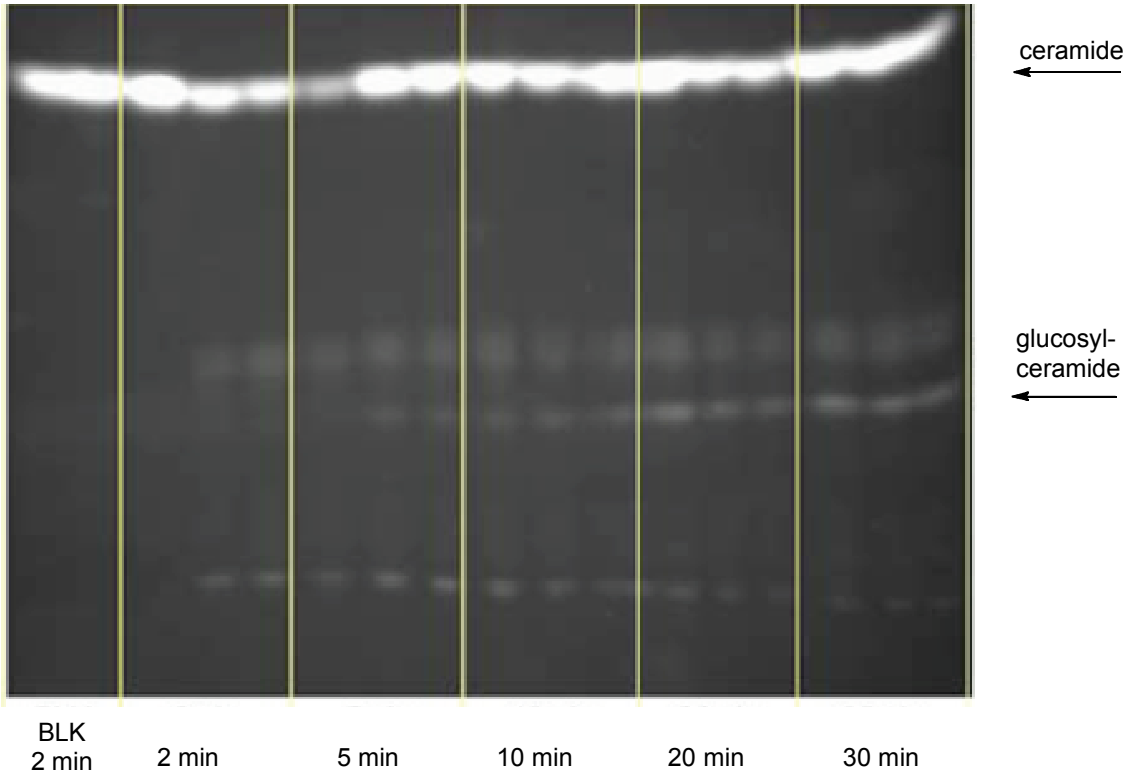
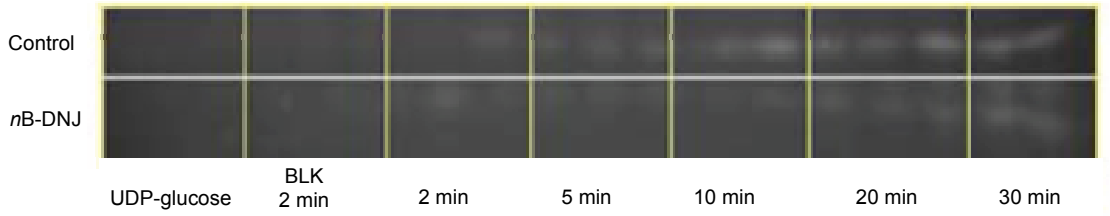


Figure 34 illustrates a parallel incubation performed with and without an inhibitor of CGT. In the presence of *n*B-DNJ, the formation of NBD-C6-*GlcCer* was clearly retarded and lower signal strength was observed at all time points.

Figure 34. Side-by-side comparison of NBD-C6-*GlyCer* produces in the absence (upper row) and the presence (lower row) of 100 μM *n*B-DNJ



IC_{50} values for *n*B-DNJ were then determined, using both mouse and rat testicular CGT, as shown in **Table 2**.

Table 2. IC_{50} of *n*B-DNJ and *n*B-DGJ on testicular CGT

	Species \ Tissue	Testis (μM)
*n*B-DNJ	LE rat	32.0 ± 25.5 (n = 4)
	C57BL/6	51.0 ± 28.0 (n = 3)
	C57BL/6 (lit.)	22.9
*n*B-DGJ	LE rat	145.0 ± 97.7 (n = 3)
	C57BL/6	> 300 (n = 2)
	C57BL/6 (lit.)	30.0

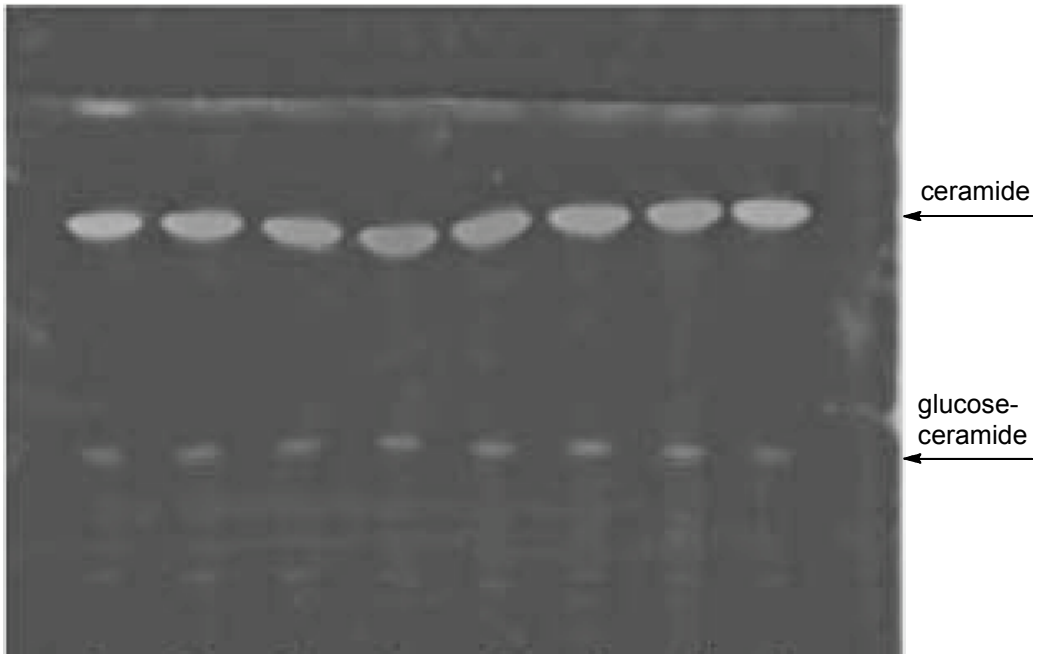
The IC_{50} for *n*B-DNJ on mouse testicular CGT was higher than the reported value,[24] but was within an acceptable range. It is interesting to note that the IC_{50} was relatively higher in the mouse microsomes in comparison to the rat. This is opposite to the relative efficacy of *n*B-DNJ with respect to its contraceptive effect, as it is known that *n*B-DNJ does not induce infertility in rats. This observation could indicate differences in bioavailability and pharmacokinetics of the compound between mice and rats. Another possibility is that CGT is not the target that leads to the contraceptive effect in the C57BL/6 male mice.

IC_{50} values for *n*B-DGJ were also determined under the same conditions. The CGT extracted from the rat microsomes also responded better to the inhibition induced by *n*B-DGJ than that obtained from the mice microsomes. In addition to that, *n*B-DGJ was less potent than *n*B-DNJ as an inhibitor, which matches the literature report.[24]

1.6.1.2 Screening of analogues as CGT inhibitors

With the IC_{50} value for *n*B-DNJ determined as the positive control, we then conducted the IC_{50} value determinations for all iminosugar analogues. However, none showed inhibition of CGT. **Figure 35** illustrates a representative image of the CGT inhibitory assay, in which no decrease of NBD-C6-*GlcCer* is observed at doses of the iminosugar analogues ranging from 0.5 to 300 μM.

Figure 35. Activity of compound **24** on C57BL/6 testicular CGT



Lane	1	2	3	4	5	6	7	8
conc. (μM)	BLK	0.5	1	10	50	100	300	BLK

1.6.1.3 Concluding remarks

A CGT inhibitory assay was developed by our collaborators because reported assay conditions were not reproducible. *n*B-DNJ was assayed against C57BL/6 and Long Evans rat testis microsomal CGT. *n*B-DNJ showed inhibitory effect on enzymes derived from both species, with a higher sensitivity to the

mouse enzyme. None of the synthetic *n*B-DNJ analogues showed inhibition of CGT in this assay up to 300 μM. The results suggest: a) steric bulk and/or aromatic substituents on the nitrogen atom are detrimental to CGT inhibitory activity; b) the 2- and/or 3-OH groups are important for CGT inhibition. Elimination of the 2-OH or inversion of the configuration of 3-OH leads to a loss of inhibitory activity; c) the negative results indicate the possibility that the iminosugars bind to targets other than CGT.

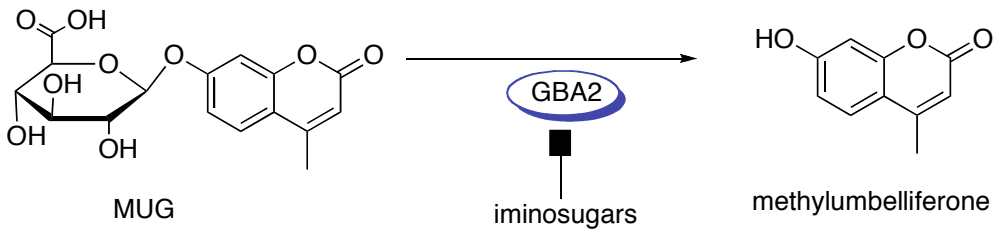
1.6.2 GBA2 inhibitory assay

GBA2 is the other proposed target that iminosugars could impact.[26] The *in vitro* biochemical assay to test the inhibitory activity of the iminosugar analogues has been optimized and standardized in Dr. Tash's group at the University of Kansas, Medical Center.

1.6.2.1 Assay development

The principle of the GBA2 assay is shown in **Figure 36**. The use of a fluorometric method provides a sensitive and quantitative assay for GBA2 inhibition. Cleavage of 4-methylumbelliferyl-β-D-glucuronide (MUG) by GBA2 yields 4-methylumbelliferone (MU), which is fluorescent above pH 8. When excited by 360 nm light, MU emits light at 460 nm. Active inhibitors of GBA2 retard the production of MU, resulting in a decrease of the fluorescent absorbance at 460 nm. Thus, the potency of the inhibitor is negatively correlated to fluorescent absorbance.

Figure 36. Principle of GBA2 inhibitory assay



The assay has been optimized for incubation time, substrate concentration and microsome concentration. Assay development has matured to the point that the Z-factor and r^2 value are 0.88 and 0.97, respectively, over an 11 minute incubation period. **Figure 37-40** show the time course of GBA2 activity in the presence of different concentrations of *n*B-DNJ.

Figure 37. GBA2 actitivity versus incubation time in the presence of *n*B-DNJ, with rat testicular microsome

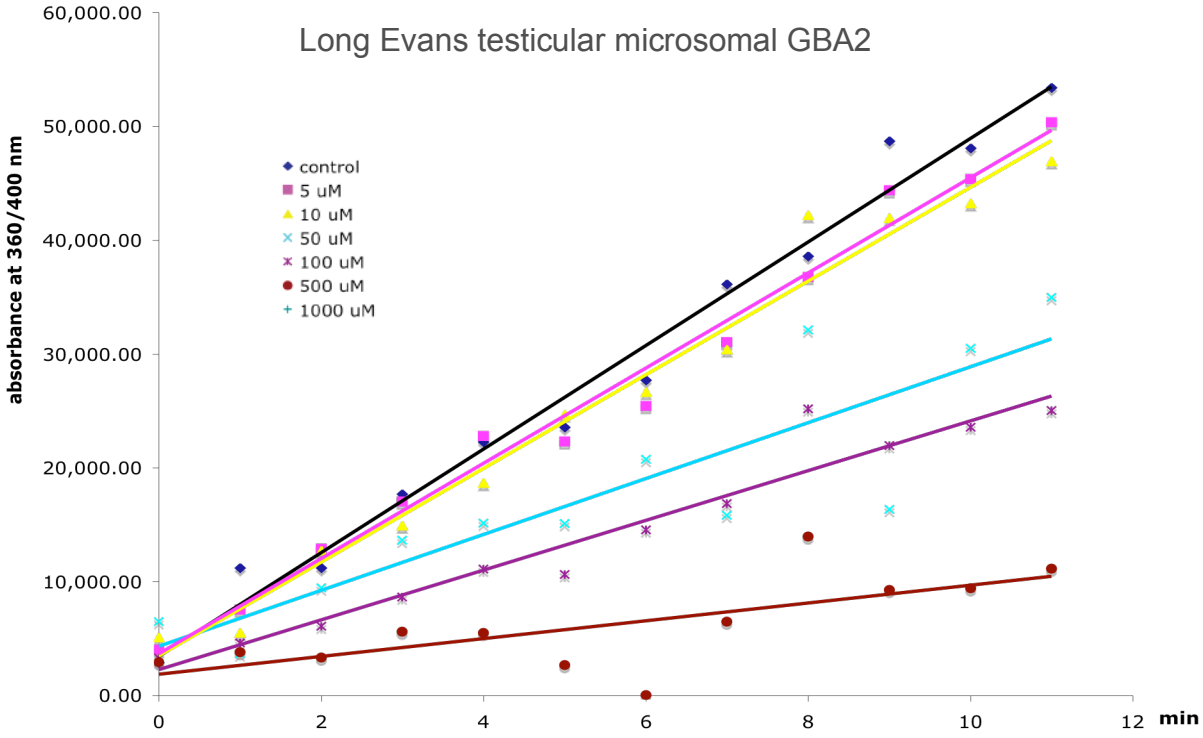
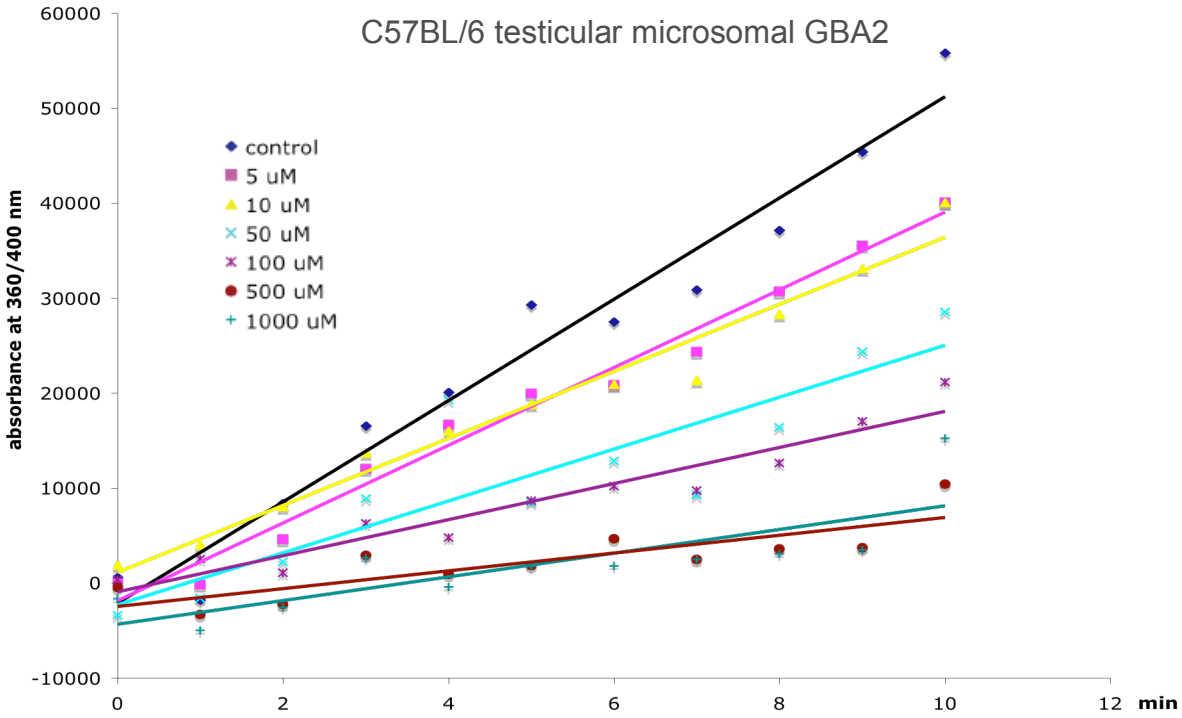


Figure 38. GBA2 actitivity versus incubation time in presence of *n*B-DNJ, with mice testicular microsome



As seen in **Figure 37** and **38**, the fluorescent absorbance declines with increasing concentration of *n*B-DNJ, indicating that *n*B-DNJ inhibits GBA2 activity, in both C57BL/6 mice and Long Evans rat testis. It is evident that C57BL/6 testicular GBA2 is more sensitive to *n*B-DNJ, when comparing the slope of the plot at 100 μM *n*B-DNJ (purple line) to that of the rat testicular GBA2 assay.

Figure 39 and **40** demonstrate the effect of *n*B-DNJ on C57BL/6 and Long Evans liver GBA2 activity. Long Evans liver GBA2 is more sensitive to the inhibition than that of C57BL/6 mice.

Figure 39. GBA2 actitivity versus incubation time in presence of *n*B-DNJ, with rat liver microsome

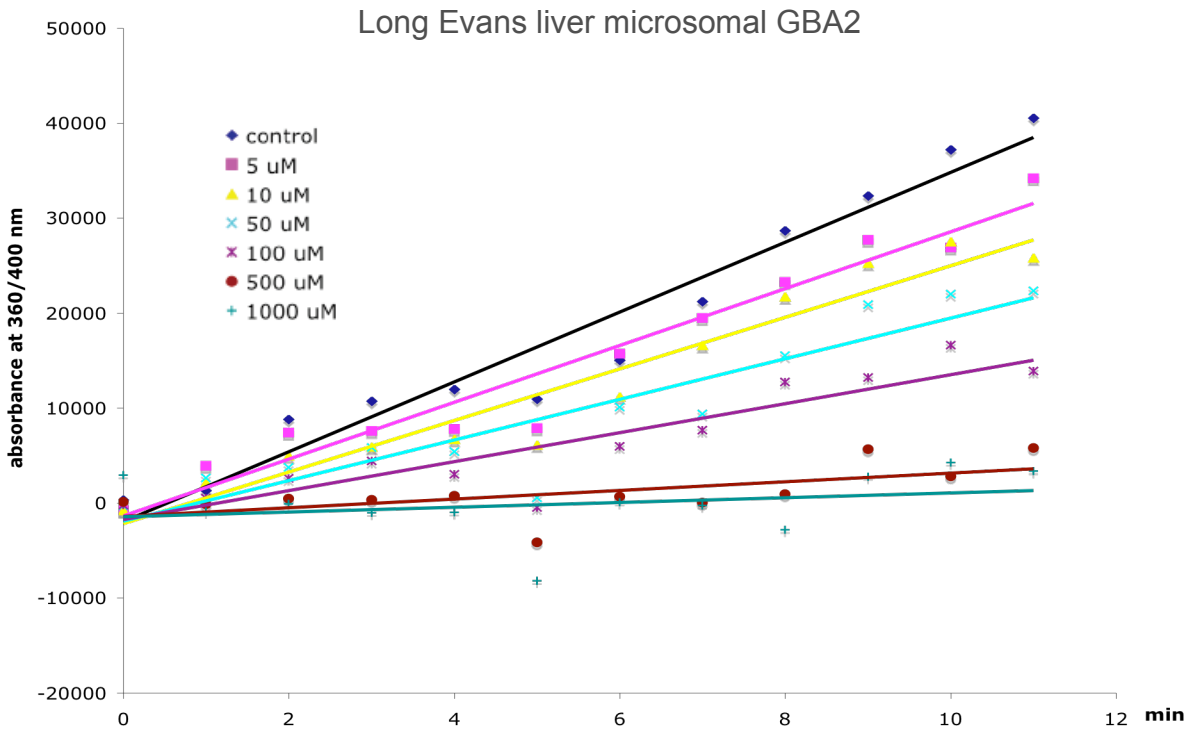
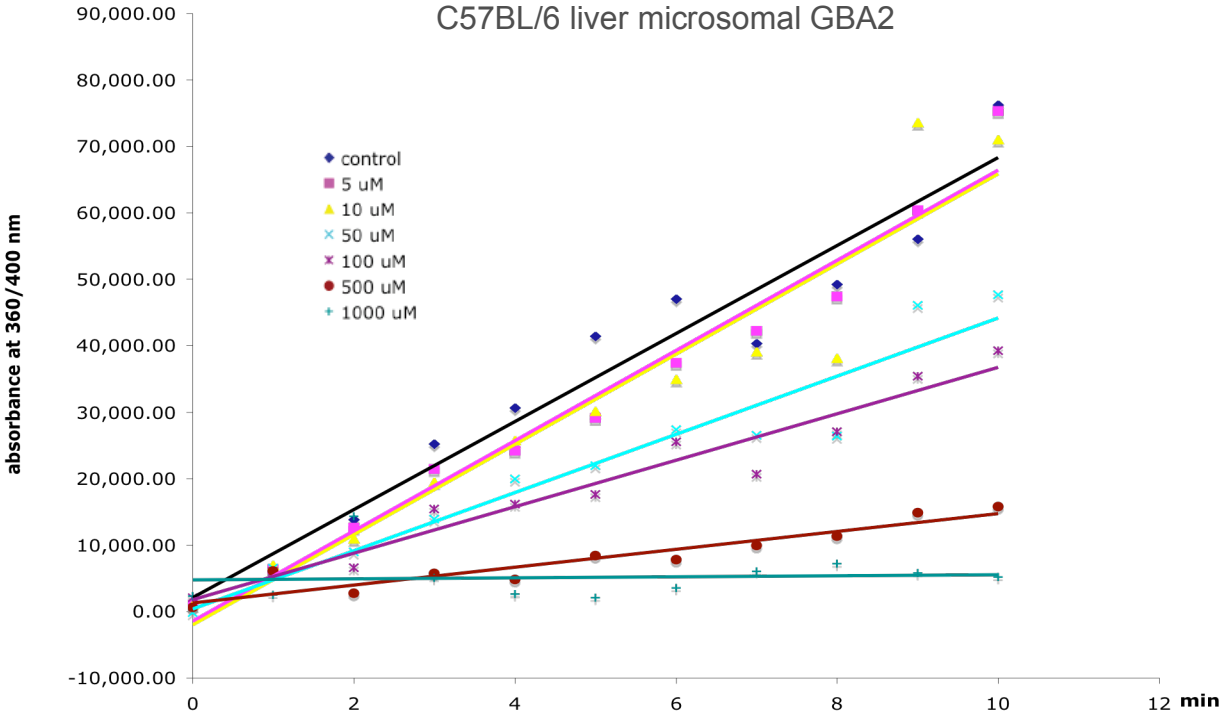


Figure 40. GBA2 actitivity versus incubation time in presence of *n*B-DNJ, with mouse liver microsome



Subsequently, the IC_{50} value of *n*B-DNJ was determined and compared to the literature data,[26] as shown in **Table 3**.

Table 3. IC_{50} of *n*B-DNJ on microsomal GBA2

Species \ Tissue	Testis (μM)	Liver (μM)
LE rat	81.2	5.0
C57BL/6	4.9	31.5
C57BL/6 (lit.)	0.14	~ 0.14

Based on these data, *n*B-DNJ appears to be a relatively potent inhibitor of microsomal GBA2 (C57BL/6 testis and Long Evans liver, especially), although the IC_{50} values from Dr. Tash's laboratory are higher than the reported ones.

Tash's assay also revealed species/tissue dependence, as the potency of *n*B-DNJ varies when GBA2 from different organs is assayed.

1.6.2.2 Concluding remarks and future directions

An *in vitro* assay for GBA2 has been set up. IC_{50} values of *n*B-DNJ have been determined using this assay, and *n*B-DNJ appears to be a relatively potent inhibitor of GBA2, which is consistent with the literature reports. Comparing the IC_{50GBA2} to the IC_{50CGT}, *n*B-DNJ appears roughly ten-fold more potent in inhibiting testicular GBA2 of C57BL/6 mice, suggesting inhibiting GBA2 might be a more effective approach to induce infertility in mice. Screening of the synthetic iminosugars is underway, and the assay results will be available in the near future.

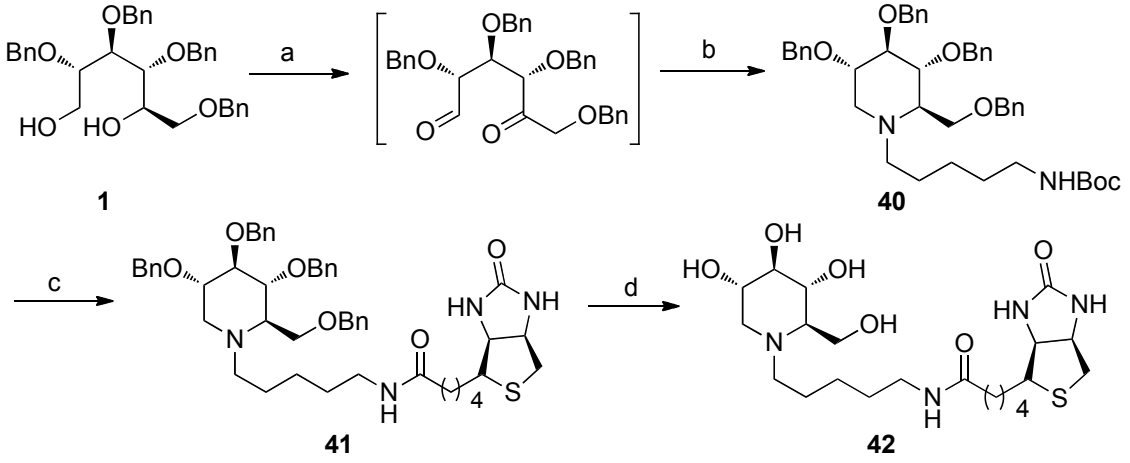
1.6.3 Affinity labeling studies

Given that the mechanism of *n*B-DNJ's species dependency unsolved for its male contraceptive effect, we hypothesized that the iminosugar could possibly interact with a unique protein target(s) expressed in C57BL/6 mice to induce male infertility. Based on our biological assay results, we postulated that this potential target is possibly different from CGT and GBA2. Therefore, affinity labeling studies using iminosugar analogues are directly pertinent to our research.

1.6.3.1 Preparation of the affinity labels

In order to isolate and characterize not yet identified targets, we prepared the affinity labels shown in **Schemes 16** and **17**. Diol **1** was oxidized to the corresponding ketoaldehyde intermediate under Swern conditions. Without isolation, the crude material was subjected to a reductive alkylation with the linker *t*-butyl-5-aminopentylcarbamate ($BocNH(CH_2)_5NH_2$). Following deprotection of the Boc group, the free amine was coupled with biotin to yield the *O*-benzylated affinity probe. The benzyl protecting groups were removed under hydrogenolysis conditions and the crude product was eluted through Dowex-50 to afford the desired DNJ-affinity label. The biotin group is used for affinity purification, so that only specific binding protein(s) can be isolated for further analysis.

Scheme 16

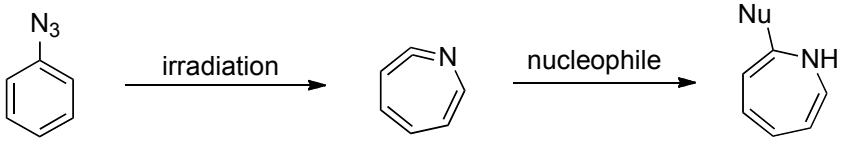


a) oxalyl chloride, NEt_3, DMSO, CH_2Cl_2; b) $BocNH(CH_2)_5NH_2$, $NaBH_3CN$, MeOH, 4Å MS, *52%*;
c) 1. 4M HCl in dioxane; 2. HATU, NMM, biotin, DMF, *92%;* d) H_2, $PdCl_2$ (4 equiv), $EtOH/H_2O$, *31%*.

A second-generation affinity label contains an aryl azide group, which crosslinks non-specifically to the side chains or backbone of the protein after the

specific binding takes place, increasing the chance of isolating iminosugar-binding protein(s).

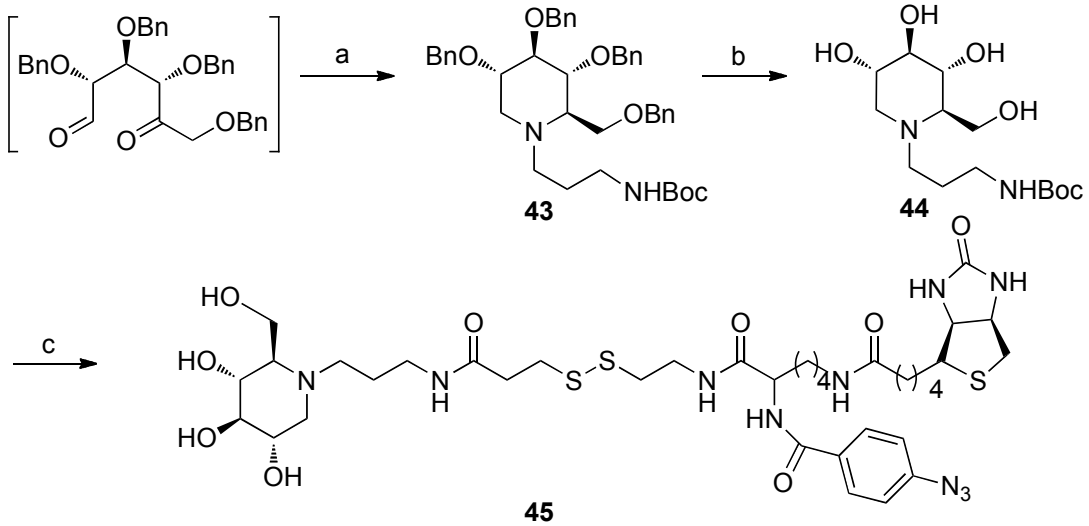
Figure 41. Mechanism of nucleophilic attack upon an irradiated phenyl azide



Azides are chemically inert compounds that upon irradiation form a highly reactive nitrene. Alkyl nitrenes generated in a solution of hydrocarbons will insert into solvent C-H bonds.[79] Aryl nitrenes, however, are far less reactive and do not react with hydrocarbons. Upon irradiation, they rearrange to a 7-membered strained heterocumulene, as shown in **Figure 41**.[80] This ketenimine intermediate can then react with nucleophiles in up to 70-80% quantum yields.

The synthesis of the photo affinity label **45** is shown in **Scheme 17**.

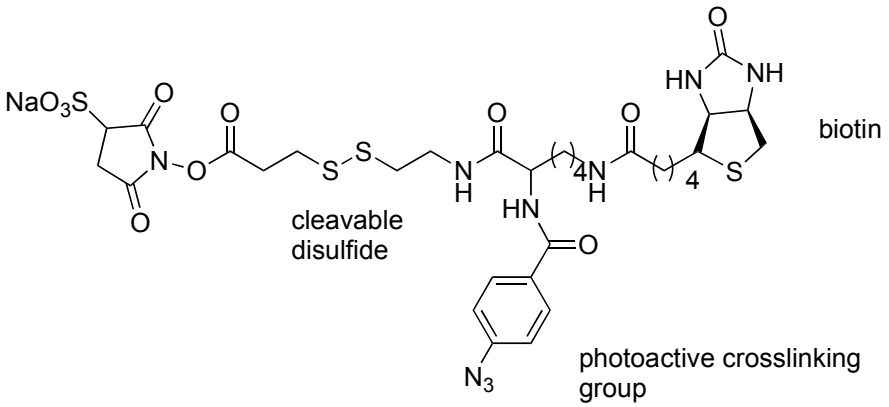
Scheme 17



a) $BocNH(CH_2)_3NH_2$, $NaBH_3CN$, MeOH, 4Å MS, *58%*; b) Pd/C, $HCOONH_2$, EtOH, reflux, *77%*; c) 1. HCOOH; 2. NEt_3, sulfo-SBED, DMSO, *95%*

t-Butyl-5-aminopropylcarbamate ($BocNH(CH_2)_3NH_2$) was selected as the linker between the sugar moiety and the affinity label. Following reductive alkylation, the *O*-benzyl protecting groups were removed under hydrogenolysis conditions. Subsequently, the *N*-Boc group was removed and the free amine was subjected to the coupling reaction with the sulfo-SBED (**Figure 42**) to yield the desired DNJ-photoaffinity label **45**.

Figure 42. Structure and functional properties of sulfo-SBED reagent

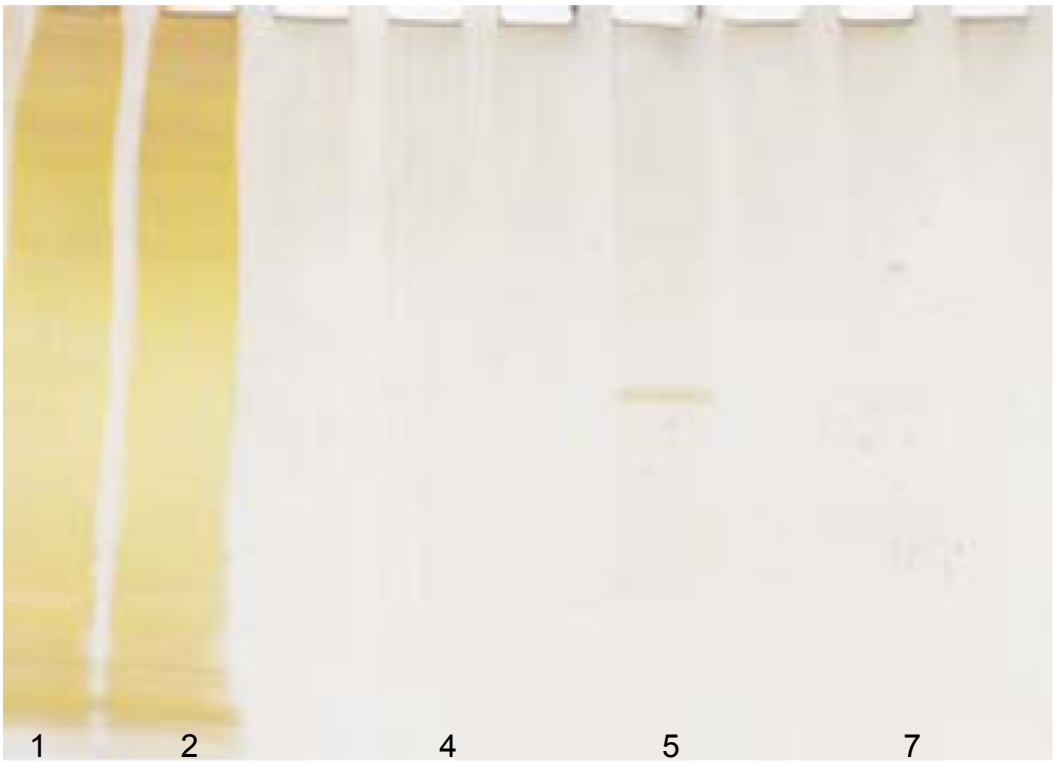


1.6.3.2 Analysis of the affinity assay

The affinity labeling studies were conducted following a protocol similar to the one that was used to identify the endogenous binding targets of gamendazole.[81] Both iminosugar affinity labels were incubated with C57BL/6 mice testicular cytosol and **45** was irradiated with UV light (254 nm) for 10 minutes. The following purification removed the non-specific binding proteins by eluting the cytosol through an avidin-agarose column. In both experiments, a protein band of ~60 kDa was isolated (electrophoresis), utilizing either affinity label, as seen in **Figure 43**. The binding of the DNJ affinity labels to this protein

could be competitively inhibited by *n*B-DNJ, indicating that *n*B-DNJ binds to this C57BL/6 cytosolic protein in a specific manner. The protein will be identified by mass spectroscopic and and peptide sequence analysis.

Figure 43. Identication of iminosugar target(s) in C57BL/6 testis



1 Rxn run-off (cytosol + BT-UV-*n*B-DNJ + DMSO
2 Rxn run-off (cytosol + BT-UV-*n*B-DNJ)
4 1.5 mM *n*B-DNJ elute (cytosol + BT-UV-*n*B-DNJ + *n*B-DNJ)
5 1.5 mM *n*B-DNJ elute (cytosol + BT-UV-*n*B-DNJ + DMSO)
7 3 mM *n*B-DNJ elute (cytosol + BT-UV-*n*B-DNJ + DMSO)

1.6.3.3 Concluding remarks and future directions

Two iminosugar affinity labels were prepared and utilized to isolate a potential protein target(s) in C57BL/6 mice for *n*B-DNJ interaction. To date, the Tash group has isolated a ~60 kDa protein from the mouse testicular cytosol,

which specifically binds to *n*B-DNJ. Mass spectroscopic and peptide sequence analysis are underway to identify and characterize this protein. Following investigations will delineate the role this protein plays in spermatogenesis and illustrate downstream "events" upon its inhibition. Therefore, we expect to have a better understanding of the mechanism that underlies the male contraceptive effect of *n*B-DNJ on C57BL/6 male mice, which could explain the species-specific activity of *n*B-DNJ and lead to the discoveries of new "druggable" targets.

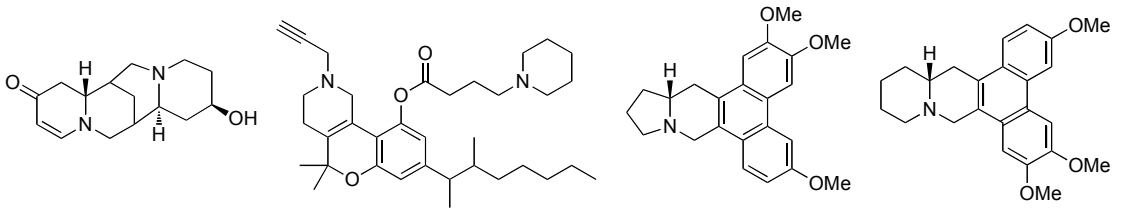
Chapter 2

Chemistry of 2,3-Dihydropyridin-4-(1*H*)-ones and Related Enaminones in Multicomponent Reactions

2.1 Background

Enaminones are excellent precursors for alkaloid natural product synthesis and can be used as building blocks for synthetic nitrogen-containing bioactive agents (**Figure 44**). This long-ago discovered, yet underexplored class of molecules demonstrates unique chemical properties that we found complementary to our interests in natural product and diversity oriented synthesis (DOS).[82,83]

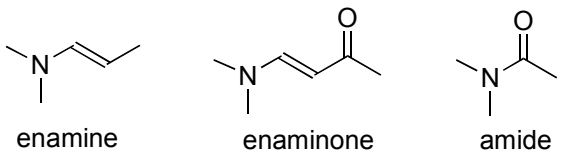
Figure 44. Representative alkaloids that can be derived from enaminones



* Total syntheses of the alkaloids and corresponding analogues have been achieved in the Georg group

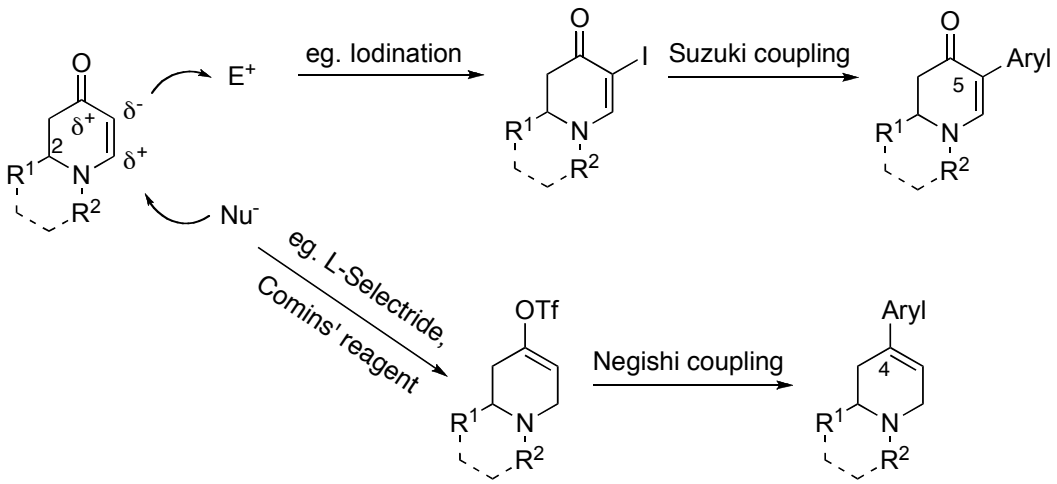
Enaminones can be best described as β-acyl enamines or amides with an interpolated alkene (vinylogous amide), as shown in **Figure 45**. Enaminones differ from enamines with respect to reactivity and stability, as the enaminone functionality is more stable against hydrolysis and oxidation than an enamine moiety. The conjugate system formed by appending the enamine moieties to a carbonyl group, attenuates its reactivity and increases its stability, although the enaminones are not quite as robust as the conventional amide.

Figure 45. Structural features of enaminone



Yet enaminones are highly polarized molecules and can be utilized in a variety of chemical transformations, as shown in **Figure 46**.

Figure 46. Known synthetic utility of enaminones

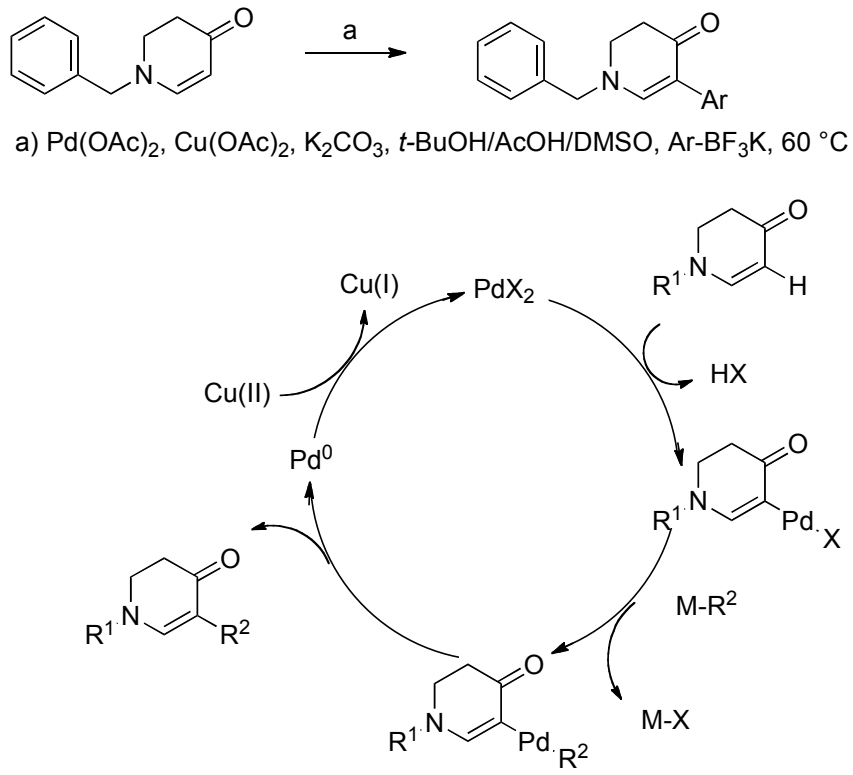


The electrophilic centers in the molecule are the carbonyl carbon (C4) and the carbon at the β-position relative to the carbonyl group (C5). A selective 1,2 reduction can be achieved under Luche conditions, and the double bond can be reduced with tri-*sec*-butyl(hydrido)borate (L-Selectride). The resulting enolate of the 1,4-addition can be trapped with Comins' reagent,[84] to afford a vinyl triflate, which is a useful synthetic intermediate that can subsequently undergo palladium-catalyzed coupling reactions to generate 4-aryl substituted piperidines. The nucleophilic sites are the basic nitrogen, and more importantly of the sp2 carbon α to the carbonyl group (C5). For instance, taking advantage of the C5

nucleophilicity, α-iodination reactions have been achieved in nearly quantitative yield under a variety of conditions.[85,86] A subsequent Suzuki-Miyaura coupling of the α-iodo intermediates with aromatic boronic acids under microwave-assisted conditions[87] afforded a wide range of 5-aryl enaminone analogues which share common structural features with many alkaloids.[88]

Recently, a more direct and concise protocol has been developed in the Georg group using organotrifluoroborates as coupling partners,[89] in order to achieve the 5-arylation of enaminones (**Figure 47**).

Figure 47. Pd(II)-catalyzed C-H functionalization with aryltrifluoroborates at C5 of enaminones



This palladium(II)-catalyzed C-H functionalization methodology takes advantage of the innate nucleophilic character of the C5 position and furnishes

the 5-aryl-enaminones in one step, thus avoiding the need for preactivation of the enaminones.

A wide variety of 5-substituted piperidine scaffolds have been constructed utilizing the powerful methodologies described above, however only aryl or alkene motifs could be successfully installed onto the enaminone C5 position. Thus, it has been our interest to develop additional efficient methodologies to furnish novel, modified enaminone scaffolds, taking advantage of the C5 nucleophilicity.

2.2 Preliminary studies concerning the C5 nucleophilicity

Several preliminary experiments were performed in order to explore the C5 nucleophilicity of enaminones.

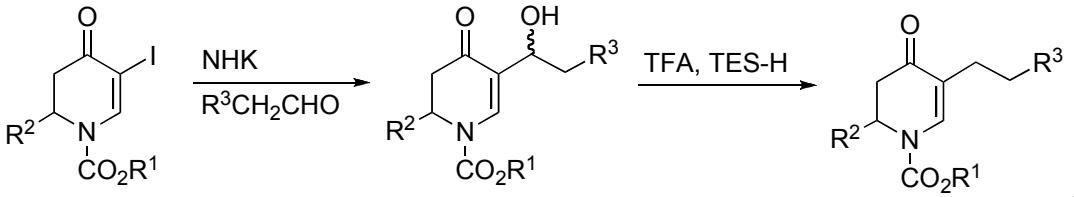
2.2.1 Installation of alkyl groups at the C5 position

Installation of a simple alkyl group at the C5 position of enaminones has not been successful, using the Pd(II)-catalyzed coupling methodology. This is mostly due to the fact that normally alkyl halides do not react with palladium. Even if the oxidative addition occurs, facile β-hydride elimination takes place prior to the desired sequence of transmetalation and reductive elimination.[90] We then set out to seek an alternative approach to prepare 5-alkyl-enaminones.

It was initially proposed to achieve our goal through an “aldol”-type reaction with aldehydes as the reaction partner, as the “aldol” formation and the following deoxygenation were both precedented in literature. Reaction of 5-iodo-

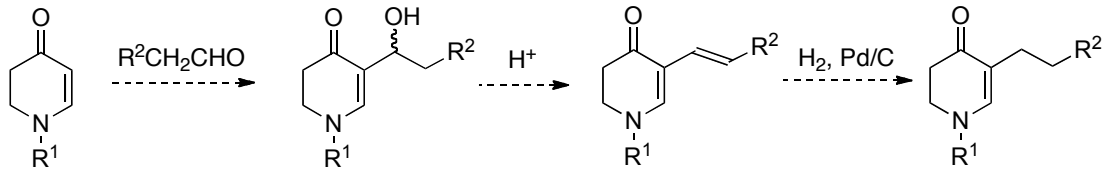
N-acyl-enaminones under Nozaki-Hiyama reaction conditions, followed by a deoxygenation[91] (**Figure 48**) furnished C5 alkyl derivatives.

Figure 48. The "aldol" approach in the literature



When comparing *N*-acyl-enaminones and *N*-alkyl-enaminones, the resonance contribution of the nitrogen is greatly enhanced in the *N*-alkyl-enaminones. In turn, the nucleophilicity of the C5 position is significantly increased and therefore it was expected that *N*-alkyl enaminones could directly attack aldehydes as depicted in **Figure 49**. The resultant α-hydroxylated intermediate could either be deoxygenated or could undergo β-elimination and hydrogenation in sequence, to complete the installation of the C5 alkyl group.

Figure 49. The "Baylis-Hillman" approach



The reaction between enaminone and aldehydes was performed as proposed above but no reaction took place under either neutral or basic conditions. Under slightly acidic conditions, rendered by the addition of TMSCl for instance, the enaminone did add to the aldehyde, as the acid activated the

aldehyde toward nuceophilic attack. However, instead of providing the desired aldol or β-elimination product, a bis-addition product was observed. Presumably the "aldol" intermediate eliminated water to afford a vinylogous imine species, as a result of the resonance contribution of the nitrogen. The newly formed vinylogous imine then reacted with a second enaminone, forming bis-addition products in good to excellent yields, as is depicted in **Figure 50** and **Table 4**.

Figure 50. C5 nucleophilicity and bis-addition product formation

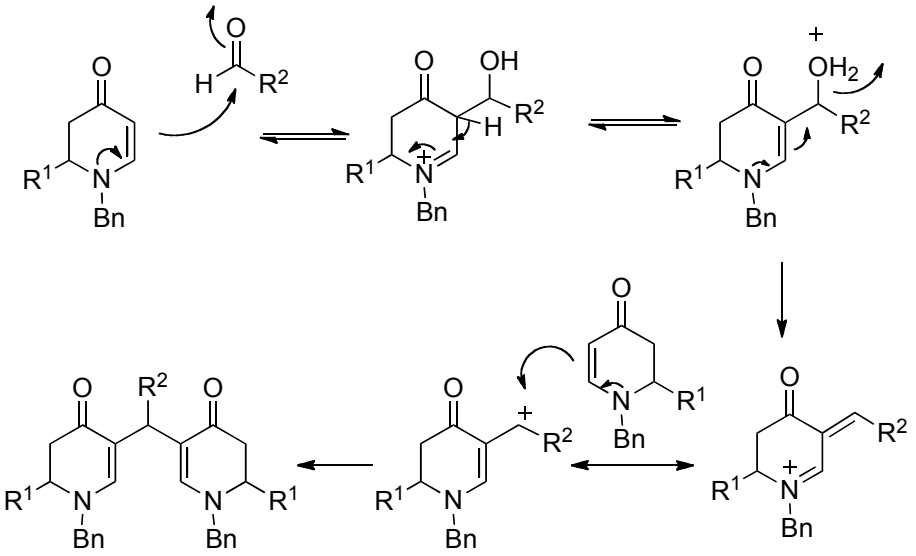
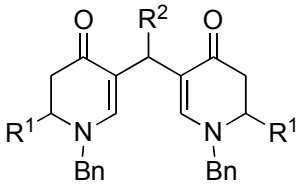
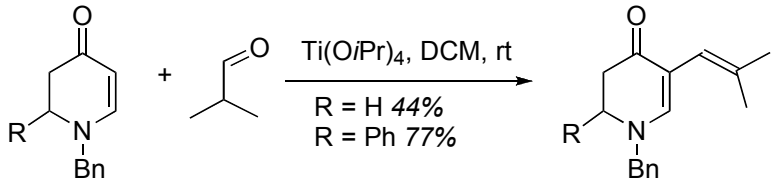


Table 4. Bis-addition products from enaminones and aldehydes

Product	R^1	R^2	Yield (%)
	H	Pr	86
	H	Ph	90
	H	2-MeC_6H_4	83
	H	4-NO_2,2-MeC_6H_3	75
	Ph	Ph	99

Attempts to “trap” the “aldol”, by lowering the reaction temperature or by adding “trapping” reagents, such as acetic anhydride and triethylsilylchloride to the reaction were unsuccessful. We also made efforts to facilitate the β-elimination pathway in order to compete with the bis-addition process. Limited success was achieved only when a sterically hindered aldehyde (isobutylaldehyde) was utilized in the presence of a metal chelating reagent ($Ti(OiPr)_4$), as shown in **Figure 51**.

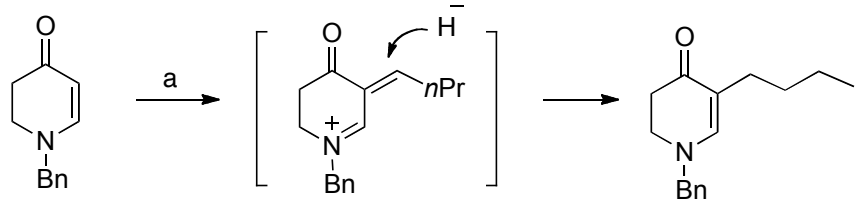
Figure 51. β-elimination pathway



Although the limited success was not synthetically useful, it demonstrated that the bis-addition process could be impeded, in this case, by steric hindrance from the gem-dimethyl groups. It was then reasonable for us to speculate that hydride delivery prior to addition of a second equivalent of enaminone, could interrupt or even block the double addition.

While some mild reducing agents, such as Pd/NH_4O_2CH and zirconocene hydrochloride (Schwartz’s reagent), were unsuccessful, the triethylsilane (TES-H) /trifluoroacetic acid (TFA) system[91] proved to be an effective hydride source to quench the vinylogous iminium species and yield the desired C5-alkylated enaminone in high yield and after a short reaction time, as shown in **Scheme 18**.

Scheme 18

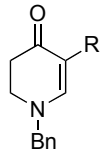
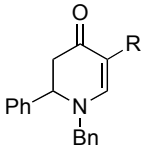
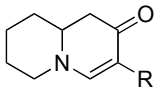
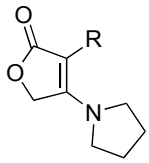


46-a

a) butyraldehyde (1.2 equiv), TFA (4 equiv), TES-H (4 equiv), DCM, reflux, 30 min, *81%*

The reaction scope of this one-flask method was then studied, and a library of 5-alkylenaminone scaffolds was prepared efficiently. The products are listed in **Table 5**.

Table 5. Library of 5-alkylenaminones

Product	Compound	R	Yield (%)
	**46a**	*n*Bu	81
	46b	Bn	85
	**46c**	*n*Bu	92
	46d	Bn	86
	46e	*n*Nonyl	77
	46f	*i*Bu	93
	**46g**	*n*Bu	88
	46h	Bn	93
	**46i**	*n*Bu	89
	46j	Bn	98
	46k	*i*Pr	88
	46l	*2*-$MeC_6H_4CH_2$	97
	46m	*4*-$ClC_6H_4CH_2$	96

High yields were obtained and no dimer formation was observed with a variety of aliphatic and aromatic aldehydes. Bicyclic enaminones were converted to the C5 alkylated products, giving comparable yields to those of the monocyclic enaminones. We also tested the reaction conditions with the (*E*)-enaminone, 4-

(pyrrolidin-1-yl)furan-2(5*H*)-one in which the nitrogen is exocyclic. Interestingly, 4-(pyrrolidin-1-yl)furan-2(5*H*)-one also underwent the transformation efficiently despite the fact that it has been reported that iodination of the β-carbon does not proceed with tertiary (*E*)-enaminones.[86]

The facile introduction of alkyl groups to C5 of enaminones allows the synthesis of a new class of enaminone derivatives. It should be feasible to introduce additional diversified substituents at the C5 position, and construct more complex enaminone derivatives.

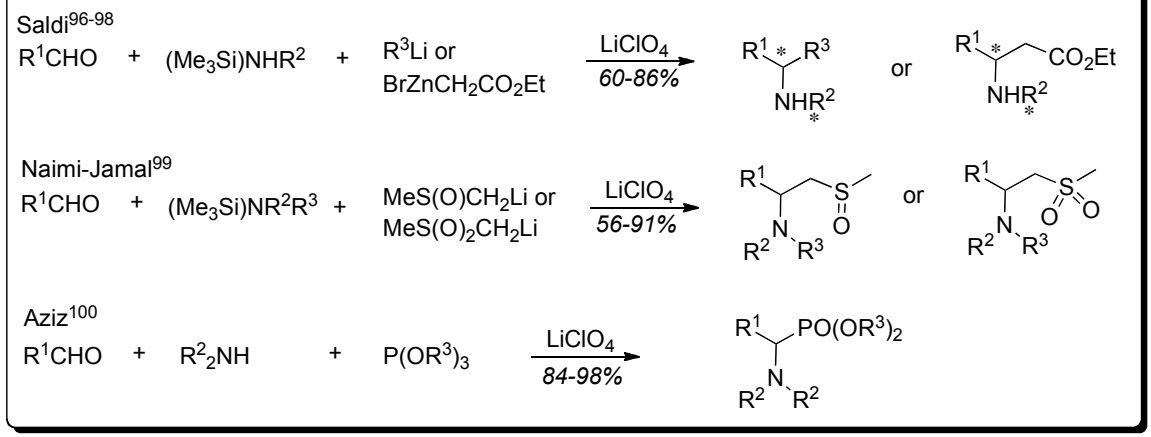
2.2.2 Aminomethylation at the C5 position and the utilization of $LiClO_4$

Encouraged by the result of the alkylation reaction, we investigated whether nucleophiles other than hydrides can participate in the three-component reaction, with enaminones and aldehydes. Amines were selected as such nucleophiles. Secondary and tertiary aminoalkylated derivatives of enaminones were expected from this Mannich reaction.[92,93] Although such reaction did proceed, it was difficult to isolate the reaction products from the undesired bis-addition products. A clean separation was only possible when preparative TLC was utilized for isolation of products.

We next resorted to acyl-protected amines (carbamates) as nucleophiles, with the expectation that the products of the multi-component reaction would be easier to isolate. When the literature was surveyed, we were able to find only one example of a carbamate participating a Mannich-type reaction.[94]

We also noticed in the literature that in the last decade, lithium perchlorate ($LiClO_4$), an inexpensive Lewis acid has been utilized often as a useful reagent to facilitate Mannich type MCR reactions as seen in **Figure 52**. $LiClO_4$ was reported to promote the Mannich reaction of electron-rich aromatic compounds with *in situ* generated iminium salts.[95] A one-flask three-component reaction with (trimethylsilyl)dialkylamine, aldehyde and a functionalized organo-lithium/zinc reagent was facilitated by $LiClO_4$, yielding a variety of secondary, tertiary alkylamines and *N,N*-dialkylamino esters in good to moderate yields.[96,97] When chiral amines were used in the one-flask reaction, high diastereoselectivity was achieved in the resultant *N*-alkylamino esters.[98] By replacing the organozinc reagents with α-lithiated salts of sulfoxides and sulfones, β-(dialkylamino) sulfoxides and β-(dialkylamino) sulfones were obtained in the presence of $LiClO_4$.[99] With trialkylphosphites as the nucleophile, tertiary α-amino phosphonates could be prepared efficiently under similar conditions.[100]

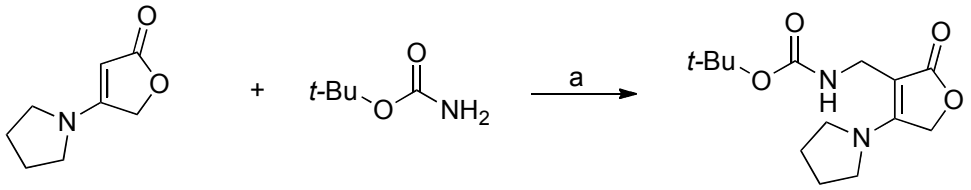
Figure 52. Examples of $LiClO_4$-mediated Mannich-type reactions in the literature



Taking into account the above literature information, we then explored $LiClO_4$ to facilitate the multi-component reaction.

Tert-butyl carbamate was selected to participate in the test reaction with an (*E*)-enaminone and formaldehyde, as shown in **Scheme 19**. We first assayed catalytic amounts of $LiClO_4$ and were pleased to observe the formation of 34% of the desired product in the presence of 50 mol% of $LiClO_4$. Higher yields were achieved when larger amounts of $LiClO_4$ were used in the reaction.

Scheme 19



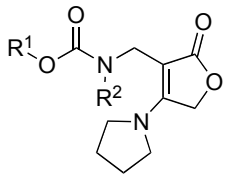
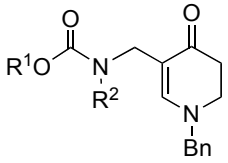
47-a

a) $LiClO_4$ (1.2 equiv), *t*-butyl carbamate (2 equiv), $(HCHO)_n$ (2 equiv), DCE, reflux, *74%*

The reaction yielded *74%* of product in refluxing dichloroethane (DCE) when 1.2 equivalents of $LiClO_4$ were added. No bis-addition product was observed. More equivalents (up to 3 equiv) of the reagents did not further affect the yield. Other lithium salts or metal chelating reagents (eg. LiCl, LiI, $Ti(O\textit{i}Pr)_4$) were also tested, but no desired product was detected.

With the standard conditions established, several carbamates were subjected to this reaction. A list of products is shown in **Table 6**.

Table 6. Library of 5-aminomethylated enaminones

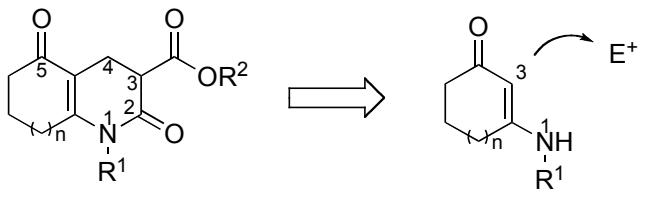
Product	Compound	R^1	R^2	Yield (%)
	**47a**	*t*Bu	H	74
	47b	Ph	H	99
	47c	Bn	Bn	86
	47d	allyl	Bn	99
	47e	Bn	allyl	74
	47f	Bn	Bu	51
	**47g**	Ph	H	54
	47h	Bn	Bn	62
	47i	Bn	*4*-$MeOC_6H_4CH_2$	51
	47j	*t*Bu	H	NR

Typically higher yields were achieved when the (*E*)-enaminone was utilized as the reactant; even quantitative yields were obtained in some cases. With the (*Z*)-enaminone lower yields were observed under the same reaction conditions. Bulky groups on the carbamates were not well tolerated with either (*E*) or (*Z*)-enaminones. When benzyl (cyclohexylmethyl)carbamate and benzyl isopropylcarbamate (bulky groups as R^2) were reacted with the (*E*)-enaminone, less than 10% of products were obtained. The (*Z*)-enaminone did not react with *t*-butyl carbamate (bulky group as R^1). Nevertheless and more importantly, we discovered in this study that $LiClO_4$ could be utilized to inhibit the adverse bis-addition reaction and facilitate the desired multi-component reaction. This method enabled us to carry out the synthesis of aminoalkylated enaminone derivatives.

2.3 $LiClO_4$-assisted synthesis of 2,5-dioxo-octahydroquinoline-3-carboxylates

We had envisioned that the exploration of the reactivity of the nucleophilic β-carbon of the enaminones could potentially lead to more complex enaminone derivatives. For instance, we envisaged that scaffolds, containing a bicyclolactam nucleus, could be assembled from the (*E*)-enaminones with electrophiles under appropriate conditions, as shown in **Figure 53**.

Figure 53. 2,5-Dioxo-octahydroquinoline-3-carboxylates



R^1 = Alkyl; R^2 = Me, Bn; n = 0, 1

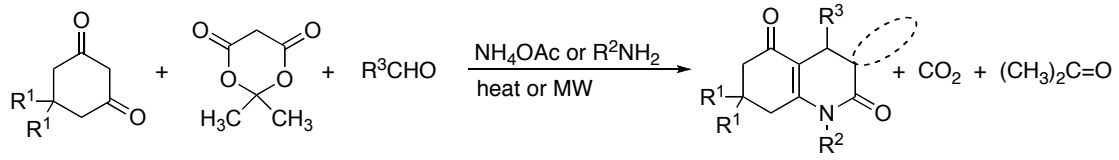
bicyclolactam

n = 0, 1

(*E*)-enaminone

Typically, a mixture of equimolar amounts of Meldrum's acid, dimedone, aldehyde, and an excess of ammonium acetate (NH_4OAc) or an amine are heated in a reaction vessel to construct such molecules.[101-104] (**Figure 54**)

Figure 54. Literature methods



R^1 = H, Me; R^2 = H, alkyl; R^3 = aryl, alkyl

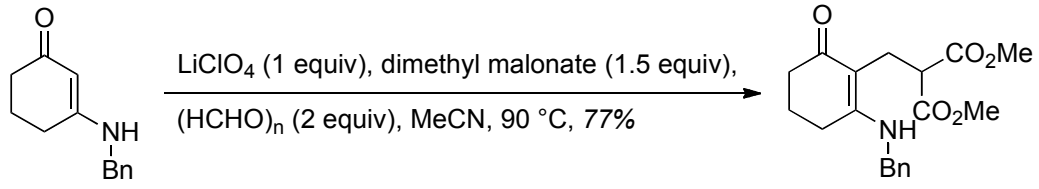
However, under such reaction conditions, the loss of one of the ester groups of Meldrum's acid (loss of CO_2 and acetone) is unavoidable. Hence, it is not possible to construct 3-carboxylate derivatives under such conditions.

Since our preliminary studies showed that $LiClO_4$ could inhibit the double addition of enaminones to aldehydes, we proposed that we could achieve a multi-component reaction similar to the aminomethylation, by modifying the corresponding reaction conditions, more specifically by using a malonate as the replacement for a carbamate. With such an adduct in hand, the C3 carboxylate could be potentially retained in the final cyclized product after a following annulation.

2.3.1 $LiClO_4$-assisted formation of enaminone methylmalonates

We then carried out test reaction where an (*E*)-enaminone (derived from the condensation of dimedone and an amine),[105] formaldehyde and dimethyl malonate were subjected to heating in acetonitrile, in the presence of one equivalent of $LiClO_4$, as depicted in **Scheme 20**. To our delight, the desired adduct was detected in a moderate yield, along with the bis-addition side product.

Scheme 20

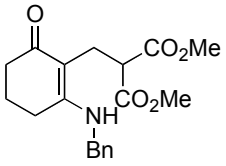


48a

Optimization of the reaction conditions showed that an equal molar amount of $LiClO_4$ was crucial and adequate to achieve a good yield. Acetic anhydride was utilized as an additive in an attempt to cyclize the MCR adduct in

one flask. Although no cyclized product was detected, we observed that the yield of the MCR reaction had improved. In addition, the presence of acetic anhydride allowed the completion of the reaction at lower temperature within a comparable amount of time, as shown in **Table 7**. Several other Lewis acids were tested, however no satisfactory results were obtained.

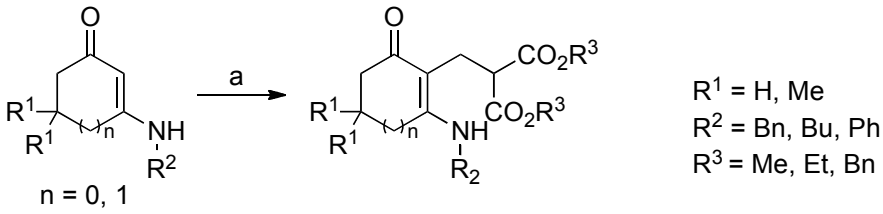
Table 7. Optimization of the $LiClO_4$-assisted reaction

Product	Temperature (°C)	$LiClO_4$ (Ac_2O) (equiv)	Yield (%)
 **48a**	90	/	12
	90	0.9	63
	90	1.0	77
	90	1.9	75
	90	10.0	62
	60	1.0 (1.0)	93*
	60	LiO_2CCF_3 / 1.0	57
	60	$Ti(OiPr)_4$ / 1.0	/ **
	60	$MgBr_2 \bullet OEt_2$ / 1.0	/ **

* used as the standard condition for following transformation;
** bis-addition product observed, no desired product formation.

Next, several enaminones and malonates were subjected to this reaction, as depicted in **Scheme 21**.

Scheme 21

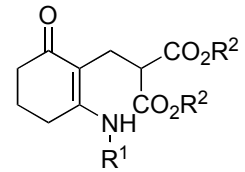
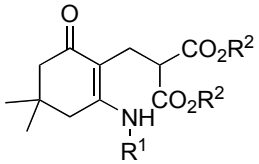
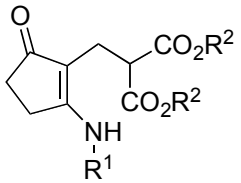


a) $LiClO_4$ (1.0 equiv), $(HCHO)_n$ (2.0 equiv), malonate (1.5 equiv), Ac_2O (1.0 equiv), MeCN, 60 °C

The reaction products were obtained in moderate to excellent yields (66%-97%) and are listed in **Table 8**. Sterically hindered malonates (eg. *tert*-butyl ethyl

malonate, dimethyl 2-methylmalonate) did not react under these conditions. Methyl acetoacetate and triethyl phosphonoacetate were tested as surrogates for malonates, but no product was detected under the standard conditions. Attempts to replace formaldehyde with other aldehydes such as benzaldehyde, were not successful.

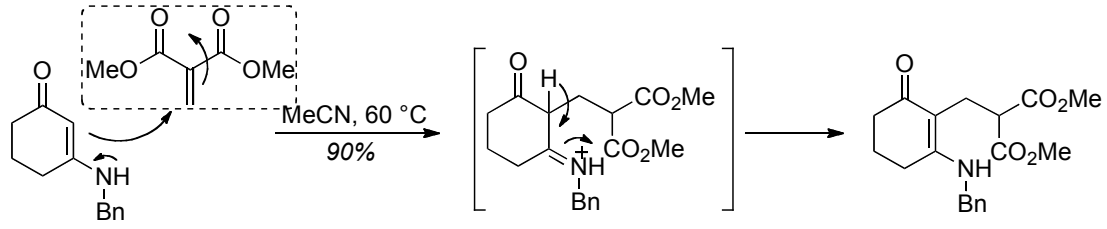
Table 8. $LiClO_4$-mediated MCR with enaminones, formaldehyde and malonates

Product	Compound	R^1 =	R^2 =	Yield (%)
	**48b**	Ph	Me	90
	48c	Ph	Bn	97
	48d	Bn	Me, Bn	73
	48e	Bn	Bn	90
	48f	*n*Bu	Me	66
	48g	*n*Bu	Bn	83
	48h	*N*-morpholine	Me	72
	48i	*N*-morpholine	Bn	86
	**48j**	Ph	Me	83
	48k	Ph	Bn	87
	48l	Bn	Me	95
	48m	Bn	Bn	88
	48n	*n*Bu	Me	88
	**48o**	Ph	Me	96
	48p	Bn	Me	94

2.3.2 Mechanistic studies

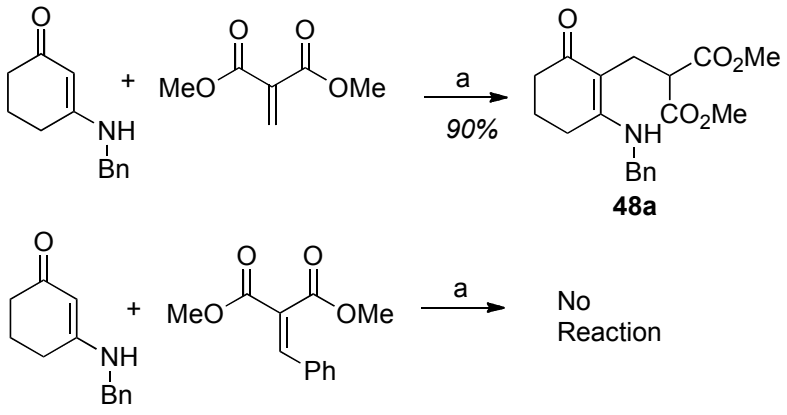
We proposed that the reaction proceeds through a Knoevenagel process producing dimethyl 2-methylenemalonate *in situ*, followed by a conjugate addition of the enaminone, as shown in **Figure 55**.[101]

Figure 55. Proposed mechanism of reaction



To confirm this pathway, the proposed intermediate dimethyl 2-methylenemalonate was prepared[106] and subjected to reaction with an enaminone, as depicted in **Figure 56**.

Figure 56. Confirmation of the reaction mechanism



a) w/ or w/o $LiClO_4$, MeCN, 60 °C

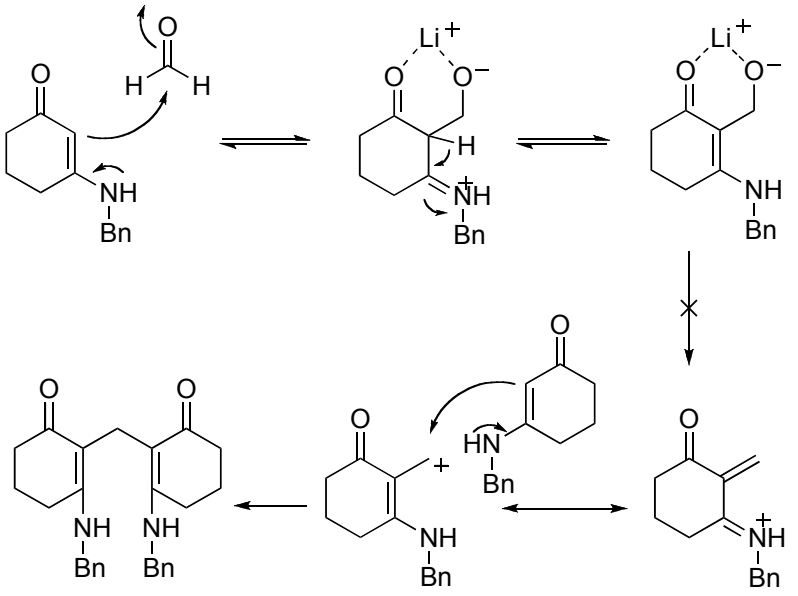
The results showed that the adduct was obtained with or without $LiClO_4$, in a comparable yield compared to that of the one-flask reaction under the standard conditions, suggesting that the $LiClO_4$-mediated three-component reaction likely proceeds through a pathway which contains a Michael receptor formation step

and a conjugate addition step in sequence. The results also revealed that $LiClO_4$ is not necessary for the Michael addition, indicating it is responsible for other aspects of this reaction.

Unlike methylenemalonate, even in the presence of $LiClO_4$, dimethyl benzylidene-malonate[107] was unreactive with the enaminone, which explains why no reaction occurred when benzaldehyde was used instead of formaldehyde.

We think that $LiClO_4$ most likely plays a dual role in this reaction: firstly it prevents the equilibrium of the reaction from going toward the direction of the bis-adduct formation; and secondly it facilitates the formation of the Michael acceptor in the one-flask reaction.

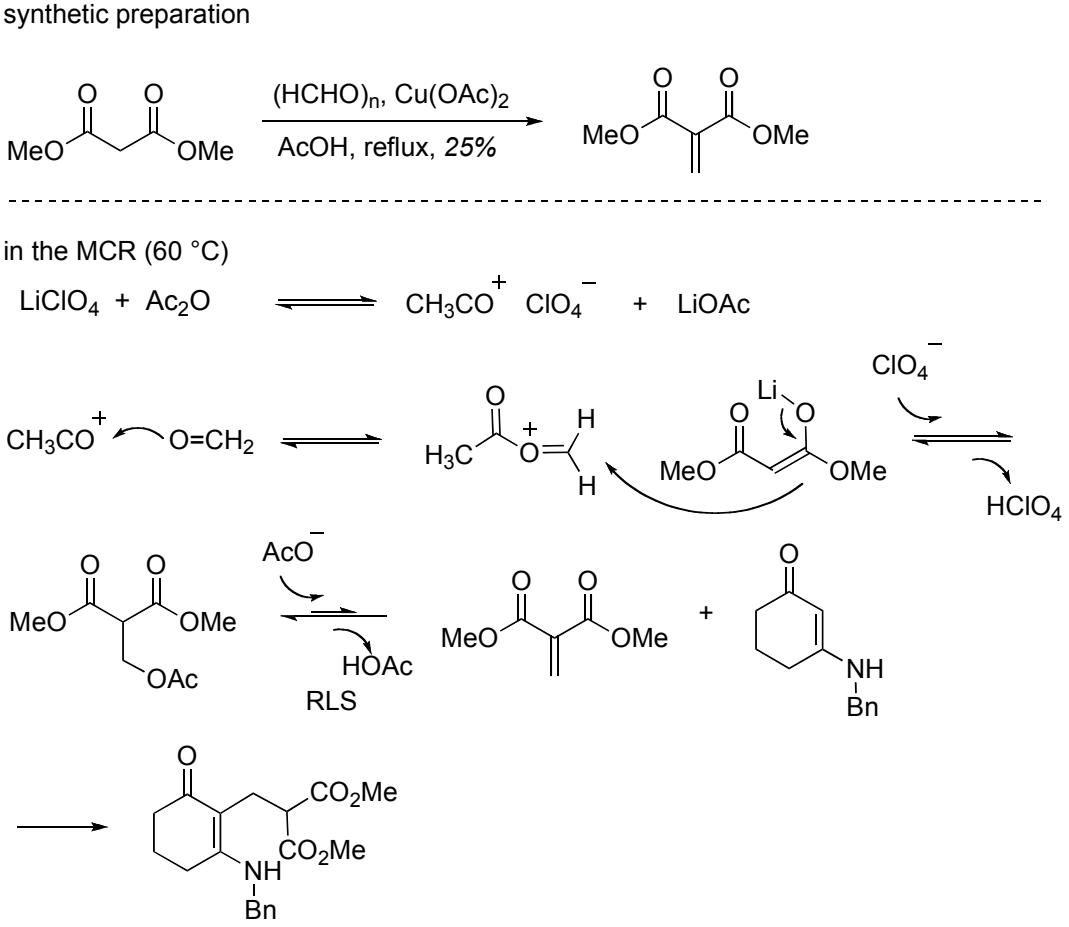
Figure 57. Proposed role of $LiClO_4$ - prevents bis-addition



The lithium cation of the weak Lewis acid presumably forms a chelate with the neighboring oxygen atoms,[108-110] which possibly lowers the energy of the ground state of the dehydration step. As a result, the formation of the iminium

intermediate, which is highly susceptible toward bis-addition by a second enaminone molecule, is largely inhibited. Therefore, the equilibrium of the reaction is shifted toward the direction of the conjugate addition in which the enaminone adds to methylenemalonate, as shown in **Figure 57**. Bivalent Lewis acids, such as Ti(O*i*Pr)$_4$ and $MgBr_2$, facilitate the dehydration step due to their stronger acidity, and therefore generate the bis-addition derivative as the major product.

Figure 58. Proposed role of $LiClO_4$ - facilitates methylenemalonate formation



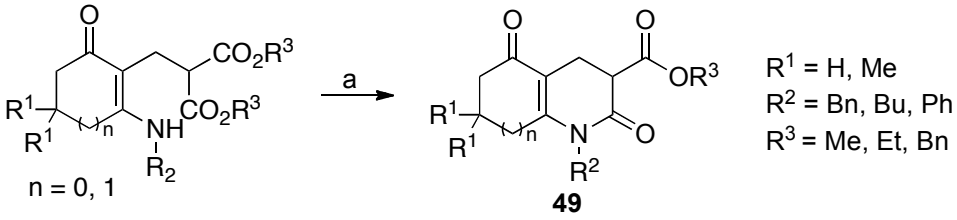
$LiClO_4$ probably also facilitates the formation of the Michael acceptor, as shown in **Figure 58**. The preparation of dimethyl methylenemalonate in a

separate flask can only be achieved under rather harsh conditions,[106] whereas the formation of the Michael acceptor in the one-flask reaction with $LiClO_4$ and the additive acetic anhydride takes place under relatively ambient conditions. It is worth noting that methylenemalonate was not detected in a control reaction to which the enaminone was not added, suggesting that elimination of the acetate is likely the rate-limiting step to yield the Michael acceptor. The presence of the enaminone possibly provides a modest amount of electrostatic stabilization to the reaction's transition state and consequently shifts the equlibrium towards the formation of the Michael addition product.

2.3.3 Annulation of the adduct

Annulation of adducts was achieved under acidic conditions,[111-113] yielding *N*-substituted 2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylates, as shown in **Scheme 22**.

Scheme 22

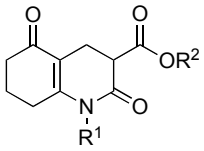
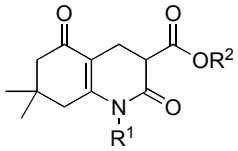
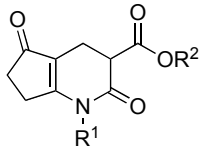


a) TFA in DCE or toluene, 90 °C, 18-24 h, *50-84*%

Adducts obtained from the three-component reaction were treated with excess trifluoroacetic acid (30 equiv) in dichloroethane (DCE) or toluene, under refluxing conditions. Cyclized products were obtained in moderate yields. A list of products is shown in **Table 9**. The cyclization of dimethylmalonate adducts were carried out in DCE; while toluene was a better choice of solvent for the

dibenzylmalonate adducts, with an aproximately 10% improvement in yields. Adducts containing a 1-morpholino group decomposed under the acidic conditions, thus cyclized products were not isolated.

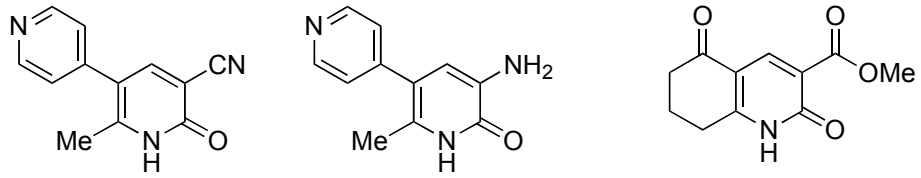
Table 9. Results of the annulation step

Product	compound	R^1 =	R^2 =	Solvent	Yield (%)
	**49a**	Bn	Me	DCE	79
	49b	Bn	Bn	toluene	70
	49c	Ph	Me	DCE	84
	49d	Ph	Bn	toluene	49
	49e	*n*Bu	Me	DCE	57
	49f	*n*Bu	Bn	toluene	60
	**49g**	Bn	Me	DCE	67
	49h	Bn	Bn	toluene	64
	49i	Ph	Me	DCE	64
	49j	Ph	Bn	toluene	51
	49k	*n*Bu	Me	DCE	62
	**49l**	Bn	Me	DCE	62
	49m	Ph	Me	DCE	49

2.3.4 Oxidation of 2,5-dioxo-octahydroquinoline-3-carboxylates

The oxidation products of bicyclolactams **49** structually resemble a class of bioactive 2-pyridone agents (**Figure 59**).

Figure 59. Bioactive 2-pyridones and derivative



Milrinone Amirinone active derivative

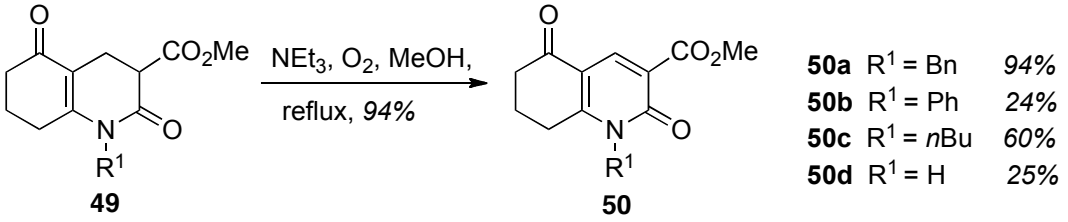
Milrinone and amirinone are prototypes of clinically useful “non-glycoside” inotropes, which are indicated for the short-term intravenous therapy of congestive heart failure (CHF).[114] The main mechanism of these agents is inhibition of the cyclic GMP-inhibited camp phosphodiesterase (type 3 PDE), resulting in an elevation of camp levels in cardiac and vascular muscles.[115] It has been suggested that the ionotropic effect of these drugs could also be attributed to the antagonism towards endogenous adenosine at the cardiac A_1 receptor.[116] Owing to their unique mechanism of action, these cardiotonic agents display a better safety profile than the conventionally used digitalis glycosides that have a narrow therapeutic index and a tendency to cause life-threatening arrhythmogenic lability.[117]

However, neither milrinone nor amirinone inhibits PDE3 or A_1 with exclusive high affinity. Thus, in a search for a new generation of cardiotonic agents with greater efficacy, these FDA approved agents have served as the prototypes of a series of analogues. Among the derivatives synthesized using milrinone as the template, methyl 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (**Figure 59**), showed ionotropic effects on the contractile force of

both guinea pig spontaneously beating atria and electronically driven left atria.[118-121]

With bicyclolactams **49** in hand, we performed an oxidation reaction in order to convert them to 2-pyridones. 2,5-dioxo-4,6,7,8-octahydroquinoline-2,5-dione-3-carboxylate was discovered to be susceptible toward oxidation under basic condition. Methyl 1-benzyl-2,5-dioxo-1,2,5,6,7,8-octahydroquinoline-3-carboxylate was converted to the oxidized product **50a** in excellent yield, upon refluxing in oxygenated methanol, as shown in **Scheme 23**. Additional analogues were obtained, however the reactions did not go to completion in all cases, therefore the yields were not satifactory.

Scheme 23



49 **50**

50a R^1 = Bn *94%*
50b R^1 = Ph *24%*
50c R^1 = *n*Bu *60%*
50d R^1 = H *25%*

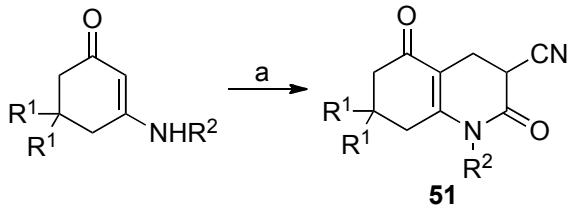
2.3.5 Synthesis of *N*-substituted 2,5-dioxo-octahydroquinoline-3-carbonitriles

Encouraged by the successful construction of enaminone methylmalonates, we attempted to expand the scope of the $LiClO_4$-assisted methodology. Different malonate surrogates were screened, although no successful reactions were achieved with malonate surrogates, such as methyl acetoacetate, triethyl phsophonoacetate and methyl cyanoacetate, using acetic anhydride as the additive.

Since it was reported that nucleophilic phosphines could be used as catalyst in internal cycloadditions to synthesize carbo- and heterocycles,[122-125] we screened phosphines as additives in our test reactions.

To our delight, with methyl cyanoacetate as a replacement for malonate, the reaction yielded the corresponding cyclized nitriles in one-flask in the presence of a catalytic amount of organophosphine. Again, formaldehyde was the only viable aldehyde (**Scheme 24**) in this reaction. A list of products is shown in **Table 10**.

Scheme 24

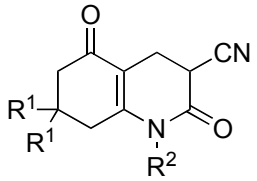


51

R^1 = H, Me
R^2 = Bn, Bu, Ph, etc.

a) $LiClO_4$ (1.0 eq), $(HCHO)_n$ (2.0 equiv), methyl cyanoacetate (1.5 equiv), PPh_3/PBu_3 (0.5 equiv), MeCN, 60 °C, 12 h

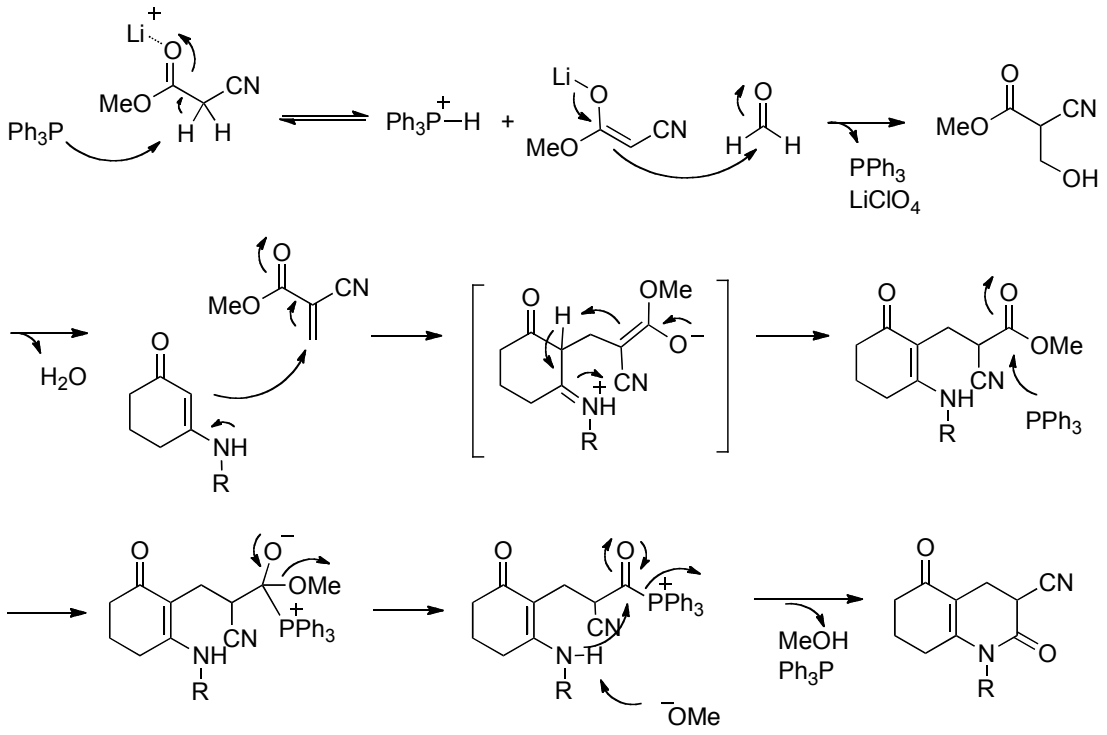
Table 10. 2,5-Dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitriles

Product	compound	R^1 =	R^2 =	Yield (%)
	**51a**	H	Bn	85
	51b	H	Ph	65 (PBu_3)
	51c	H	Bu	73 (PBu_3)
	51d	Me	Bn	68
	51e	Me	Pr	75
	51f	Me	$MeO(CH_2)_2$-	67

In this reaction no intermediate adduct was detected, suggesting that the phosphine-catalyzed Knoevenagel process[126] was directly followed by an intramolecular amide forming step.

A proposed formal [3 + 3] mechanism for the synthesis of the 2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitriles is outlined in **Figure 60**.

Figure 60. Proposed mechanism for the formation of 2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitriles



$LiClO_4$ initiates the C-C bond formation between the aldehyde and methyl cyanoacetate with the assitance of phosphine. The Michael acceptor is then formed upon elimination of the hydroxyl group. The enaminone, which is stabilized by $LiClO_4$, attacks the Michael acceptor providing the adduct. It is likely that the phosphine catalyst participates in the following intramolecular

amidation/cyclization,[127] giving the 2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitrile.

Malonates were also subjected to the reaction conditions shown in **Scheme 24**. However, no cyclized product was detected after 12 h. The bis-addition product was found to be the major product, accompied by only a small amount of the Michael addition product. This result was not very surprising in that it was reported that Knoevenagel products were not obtained from malonates and aldehydes under phosphine-catalyzed conditions.[126] As a result, the Michael acceptor could not be generated *in situ*, and the subsequent formation of enaminone methylmalonates could not take place.

2.4 Concluding remarks

In this chapter, we examined the nucleophilicity and chemical reactivity of enaminones. A practical method was discovered to install alkyl groups at the C5 position, and a $LiClO_4$-assisted aminomethylation reaction was achieved taking advantage of the C5 nucleophilicity. Susequent studies allowed us to develop a $LiClO_4$-mediated two-step synthesis of 2,5-dioxo-octahydroquinoline-3-carboxylates. This facile method provides an operationally easy approach to synthesize a class of compounds, which are potentially ionotropically active for the treatment of congestive heart failure disease. An organophosphine cataylized reaction was developed to construct 2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitriles.

Chapter 3

Experimental Data

3.1 Material and Methods

Proton nuclear magnetic resonance spectra were recorded using a 400 MHz or 500 MHz spectrometer. Carbon nuclear magnetic resonance spectra were recorded using a 100 MHz or 125 MHz spectrometer. All Chemical shifts were recorded as parts per million (ppm), and all samples were dissolved in $CDCl_3$ using residual solvent peak as internal standard unless otherwise noted. Mass spectra were obtained from a ZAB HS mass spectrometer equipped with a 11/250 data system. Fast-atom bombardment mass spectrometry (FAB-MS) experiments were performed with a Xenon gun operated at 8 Kev energy and 0.8 mA emission at the MS laboratory at the University of Kansas and the Institute for Therapeutic Discovery and Development at University of Minnesota. Fast-atom bombardment high resolution mass spectra (FAB-HRMS) were recorded at 1:10,000 resolution using linear voltage scans under data system control and collected in a multi-channel analyzer mode (MCA). A Recording Infrared Spectrophotometer or a FT-IR was used to record infrared spectra. Optical rotations were obtained using a polarimeter at room temperature. Melting points are uncorrected. All moisture-sensitive reactions were performed using either oven or flame dried glassware under a positive pressure of argon unless otherwise noted. Solvents and reagents that are commercially available were used without further purification unless otherwise noted. Tetrahydrofuran and diethyl ether were freshly distilled from sodium benzophenone ketyl under argon.

Methylene chloride was distilled freshly from calcium hydride under argon. Silica gel (230-400 mesh) used for column chromatography. All compounds were concentrated using a standard rotory evaporator and high-vacuum techniques.

3.2 Biological Procedures

3.2.1 Microsome preparation from mouse and rat testes

Testes in 5 g batches were placed in a 50 mL culture tube. To the tube, reagent A (antipain (20 µL, 1 mg/mL), leupeptin (20 µL, 1 mg/mL), aprotinin (200 µL, 1 mg/mL), APMSF (110 µL, 1 mg/mL), KCl (372 mg), deionized H_2O (18.5 mL)) and reagent B (25 mL, 0.5 M Tris, 2.0 M sucrose) were added. The testes were minced with scissors then blended by 2-3 10 sec bursts at a time on Power Gen 700 (Fisher Scientific) at setting 5-6 while on ice. The homogenate was centrifuged at 7500 rpm for 10 min at 4 ºC using a SW28 rotor. The resultant supernatant was collected and centrifuged at 23500 rpm for 1 h at 4 ºC in a SW40 rotor. The supernatant was discarded and the pellet containing the microsomes was suspended in Reagent C (600 µL, 200 mM DTT, 0.1 M EDTA, 10 mM UDP-glucose and 10% CHAPSO) and dispersed by passing through a 25-gauge needle followed by an insulin needle. The microsome suspension was stored as 100 µL aliquots in microcentrifuge tubes, flash frozen in liquid nitrogen for 1-2 min, and kept at -80 °C, and used as needed.

3.2.2 Ceramide-specific glucosyltransferase (CGT) assay

The Enzymatic Reaction: The following solutions were added to each tube: reagent A (295 μL, pH 7.4, containing 50mM HEPES, 5 mM $MnCl_2$, 10mM phosphatidylcholine, 50 uM CBE, 1 mM EDTA and 10 mM UDP-Glucose), H_2O (145 μL), iminosugar solution (50 μL) and testicular microsomes (100 μg). Control tubes contained the same components except microsome. Reactions were initiated by the addition of BSA-ceramide (3 μL), and incubated at 37 °C for 30 min, then terminated by addition of chloroform:methanol (1 mL, 2:1, v/v), vortexed and incubated at room temperature for 30-60 min to allow phase separation. The upper phase and the mid-layer were removed and discarded, and chloroform:methanol:water (500 μL, 3:48:47, v/v/v) was added to the bottom layer, vortexed and allowed to sit for 15 min at room temperature. The resultant upper phase was again removed and chloroform:methanol (100 μL, 2:1, v/v) was added and then sample tubes were dried in a vortex evaporator overnight.

Thin layer chromatography (TLC): TLC plates were pre-treated (Whatman silica gel 60 A, 20x20 cm, layer thickness 250 μm) by immersion in chloroform:methanol:water (50:50:15, v/v/v) for 5 min, air dried for 10 min, then immersed in 5% sodium borate (prepared in methanol) for 1 min, dried and heated at 120 °C for 1.5 h. The dried sample tubes were reconstituted with chloroform:methanol (100 μL, 2:1, v/v) and vortexed, a sample (20 μL) was then spotted onto the plates at the origin. The spotted plates were air-dried and placed in a sealed TLC chamber saturated with of chloroform:methanol:water

(60:30:5, v/v/v) and run for approximately 1 h until solvent reached < 1 cm from top of plate.

Detection and quantitation of substrate/product: The TLC plate was documented using UV transilluminator (302 nm) and analyzed using AlphaEase (Fluorchem SP) software. The IDV values were plotted against iminosugar concentration using Sigma Plot 10. A linear regression plot was used to determine IC_{50} values.

3.2.3 Non-lysosomal *β-glucosidase 2* (GBA2) assay

The inhibitory effects of *n*B-DNJ derivatives on the activity of the non-lysosomal glucosylceramidase were determined in membrane preparations from various tissues. Pooled mouse tissues were homogenized in water (1:3, w/v) using a Polytron PT1000 homogenizer (VWR) for 1 min. Homogenates were centrifuged for 20 min at 15,000 g (4 °C). The supernatant was removed and the pellet was resuspended in ice-cold 50 mM potassium phosphate buffer, pH 5.8. Membranes were washed three times in the phosphate buffer, resuspended in this buffer, and stored at -80 °C.

For the enzyme assay aliquots of the membrane suspension were preincubated with conduritol epoxide (CBE) (Toronto Research Chemicals, Downsview, ON, Canada) at a final concentration of 2.5 mM for 15 min, supplemented with an iminosugar to the desired concentration, incubated for 15 min, then diluted 3-fold in 4.5 mM 4-methylumbelliferyl-D-glucoside (Sigma) in 0.1 M citric acid, 0.2 M disodium hydrogen phosphate, pH 5.8, in a final volume

of 30 µl, and incubated at 37 °C for 1 h. The reaction was stopped by adding 200 µl of 500 mM sodium carbonate, pH 10.7. Released 4-methylumbelliferone was detected using a Fluoroskan Ascent fluorometer (Thermo Electron Corp., Basingstoke, Hampshire, UK; excitation 355 nm, emission 460 nm). The IDV values were plotted against iminosugar concentration using Sigma Plot 10. Linear regression plot was used to determine IC_{50} values.

3.2.4 Affinity labeling study

Incubation: A light-proof vessel containing pre-cleared cytosol (2.7 mL), affinity label (**42** or **45**, 150 µL, 10 mM stock solution diluted 1:1000) and *n*B-DNJ solution (150 µL, 5.2 mg/mL) was incubated at 4 °C in a dark room. As a control, pre-cleared cytosol (2.7 mL), affinity label (150 µL) and DMSO (150 µL) were incubated on the side.

Irradiation of the Affinity label: The sealed vessel was exposed to a UV light (254 nm) suspended at 10 cm height for 20 min. The irradiated mixture was then loaded onto an avidin D agarose column.

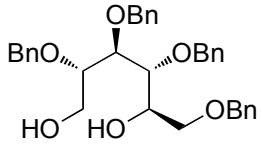
Separation: The column was washed until background was reached and was subsequently eluted with 1.5 mM *n*B-DNJ, 3 mM *n*B-DNJ, 250 mM NaCl and 600 mM NaCl in sequence. Absorbance of all eluates measured and concentrated to be resolved on 4-20% Tris HCl gel.

Silver Stain: Silver stain is compatible with mass-spectrometry analysis and has high signal to noise ratio. The gel was fixed with 50% MeOH, 12% acetic acid, 0.05% formalin, 2 h or overnight. The gel was washed with 35% EtOH for (20 min × 3). The gel was subsequently subjected to sensitization with 0.02% $Na_2S_2O_3$ for 2 min, followed by washing with H_2O (5 min × 3). The gel was then stained with 0.2% $AgNO_3$, 0.076% formalin for 20 min. Next, the gel was rinsed with H_2O (5 min × 2), and developed with 6% Na_2CO_3, 0.05% formalin and 12% acetic acid for 5 min. The gel was left at 4 ºC in 1% acetic acid.

Preparation of samples from gel bands for Mass-spectrometry study: The gel was destained till a clear background was observed. Gel bands were excised precisely and placed into a washed, plain 1.5 mL Eppendorf tube; a band from a blank area was also collected as the control. The gel slice was washed with 50% HPLC Grade acetonitrile/H_2O twice for 10 min with occasional vortex mixing. The Eppendorf tube was closed tightly after rinsing the cap off with the 50% HPLC Grade acetonitrile/H_2O solution. The samples were then submitted for mass-spectrometry analysis.

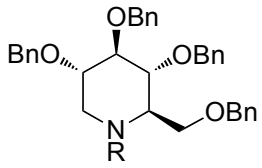
3.3 Experimental Procedures

3.3.1 Chapter 1



(2*S*,3*R*,4*R*,5*R*)-2,3,4,6-Tetrakis(benzyloxy)hexanes-1,5-diol (1). The starting

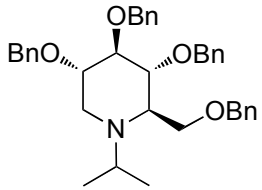
material, 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (2.0 g, 3.7 mM) was dissolved in THF (20 mL) and then $LiAlH_4$ (505 mg, 12.6 mmol) was added to the solution in portions over 20 min, at 0 °C. After the reaction had warmed to room temperature, the reaction was quenched utilizing the 1+2+3 method: for each of 1 g $LiAlH_4$ used, H_2O (1 mL) was added slowly, then 10% aqueous NaOH (2 mL), and lastly H_2O (3 mL) were added. The aluminum salts were easily filtered off on a Celite plug. The filtrate was subjected to rotary evaporation and flash column chromatography on silica gel, using 10% EtOAc:hexanes (v/v) as the eluent, to give a colorless syrup (95%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.27 (m, 20H), 4.6 (m, 8H), 4.02 (m, 1H), 3.89 (dd, *J* = 3.6, 6.2 Hz, 1H), 3.63 (d, *J* = 3.8 Hz, 2H), 3.55 (dd, *J* = 4.5, 11.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 138.3, 138.0, 128.6, 128.3, 128.2, 128.1, 128.0, 127.1, 79.6, 79.2, 77.5, 74.7, 73.6, 73.4, 73.2, 71.3, 70.8, 62.0; ESI-HRMS: calc'd *m/e* for [M+Na^+] $C_{34}H_{38}NaO_6$: 565.2566, found 565.2568; IR (neat, NaCl, cm^{-1}): 3545, 2923, 2870, 1720, 1496, 1453, 1396, 1358, 1273, 1210, 1073, 1070, 1027, 735, 698; $[\alpha]_D^{25}$ 6.8 (*c* = 0.99, $CHCl_3$).



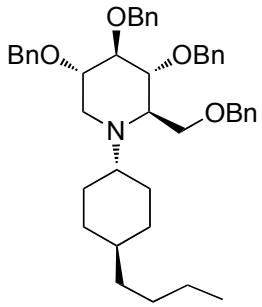
General Procedure for the Synthesis of (2*R*,3*R*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)piperidines 2. A reaction vessel, containing dimethyl sulfide (DMSO, 6.2 equiv) dissolved in CH_2Cl_2 at a concentration of 0.6 mL/mmol,

approximately, was cooled to -78 °C in a dry ice-acetone bath. A solution of oxalyl chloride (4.4 equiv) in CH_2Cl_2 (0.2 mL/mmol) was subsequently added dropwise to the DMSO/CH_2Cl_2 solution. The reaction was stirred at -78 °C for 30 min. Diol **1** was dissolved in CH_2Cl_2 at a concentration of 4 mL/mmol approximately, and the solution of the diol was added slowly to the reaction. The reaction was kept at -78 °C for 1.5 h, followed by the dropwise addition of triethylamine (8 equiv) in CH_2Cl_2 (0.5 mL/mmol), and was allowed to warm to room temperature gradually. Solvent and excess triethylamine were evaporated *in vacuo* at 40 °C. The crude amorphous solid material was used in the double reductive alkylation without further purification.

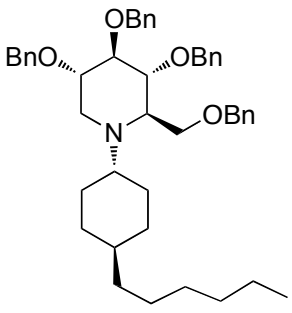
The above material was dissolved in MeOH (6 mL/mmol) and 4Å molecular sieves (0.4 g/mmol) were added. A solution of the amine (3 equiv) in MeOH was then added to the reaction. Next, sodium cyanoborohydride (2.5 equiv) was added, and the pH of the reaction was kept < 7 by adding acetic acid. (Caution: good ventilation is mandatory for the highly toxic HCN gas that is generated.) The reaction was stirred at 50 °C overnight, and quenched with 1 M NaOH. The reaction mixture was filtered through a Celite plug and diluted with H_2O (50 mL/mmol). The aqueous solution was then extracted with CH_2Cl_2 (20 mL × 2). The organic layers were combined, dried over $MgSO_4$ and then concentrated *in vacuo*. The *N*-alkylated products were purified via flash column chromatography on silica gel, using 20% EtOAc:hexanes (v/v) as the eluent.



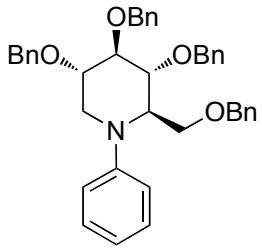
(2*R*,3*R*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)-1-isopropylpiperidine (2-1). After chromatographic purification, a viscous yellow oil was obtained (60%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.74 - 7.27 (m, 18H), 7.12 - 7.10 (m, 2H), 4.96 (d, *J* = 11.2 Hz, 1H), 4.86 (d, *J* = 10.9 Hz, 1H), 4.81 (d, *J* = 11.2 Hz, 1H), 4.68 (d, *J* = 10.3 Hz, 2H), 4.47 (d, *J* = 3.2 Hz, 2H), 4.42 (d, *J* = 10.9 Hz, 1H), 3.69 (dd, *J* = 8.1, 2.4 Hz, 1H), 3.63 (m, 1H), 3.57 (m, 2H), 3.45 (t, *J* = 9.0 Hz, 1H), 3.26 (t, *J* = 9.0 Hz, 1H), 3.07 (dd, *J* = 6.2, 4.8 Hz, 1H), 2.37 (m, 1H), 1.96 (t, *J* = 10.8 Hz, 1H), 1.08 (d, *J* = 6.7 Hz, 3H), 0.80 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 138.61, 138.55, 138.19, 138.12, 128.38, 128.35, 128.10, 127.97, 127.87, 127.77, 127.63, 127.59, 104.71, 84.66, 82.34, 77.89, 75.68, 75.02, 74.87, 74.75, 73.50, 68.95, 57.10, 29.70; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{37}H_{44}NO_4$: 566.3270, found 566.3302; IR (neat, NaCl, cm^{-1}): 3062, 3030, 2924, 2863, 1724, 1604, 1583, 1497, 1453, 1360, 1312, 1274, 1212, 1070, 1028, 910, 804, 735, 697. $[\alpha]_D^{25}$ 11 (*c* = 0.65, $CHCl_3$).



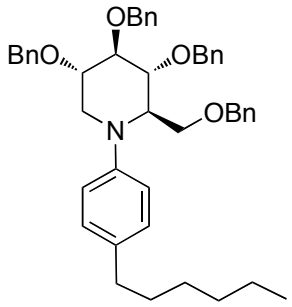
(2*R*,3*R*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)-1-((1*s*,4*R*)-4-butyl-cyclohexyl)piperidine (2-2). After chromatographic purification, a colorless oil was obtained (55%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.36 - 7.27 (m, 18H), 7.19 (d, *J* = 1.8 Hz, 1H), 7.17 (s, 1H), 4.95 (d, *J* = 11.3 Hz, 1H), 4.89 (d, *J* = 10.9 Hz, 1H), 4.82 (d, *J* = 11.3 Hz, 1H), 4.66 (ddd, *J* = 6.2, 6.2, 6.2 Hz, 2H), 4.60 (d, *J* = 12.3 Hz, 1H), 4. 51 (d, *J* = 10.9 Hz, 1H), 4.32 (d, *J* = 12.3 Hz, 1H), 3.63 (m, 1H), 3.60 (m, 2H), 3.49 (m, 1H), 3.43 (t, *J* = 9.0 Hz, 1H), 3.14 (dd, *J* = 4.8, 11.0 Hz, 1H), 2.63 (t, *J* = 11.6 Hz, 1H), 2.42 (d, *J* = 9.5 Hz, 1H), 2.02 (t, *J* = 10.7 Hz, 1H), 1.80 (d, *J* = 12.6 Hz, 1H), 1.53 - 1.26 (m, 14H), 0.96 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 139.21, 138.63, 137.70, 128.69, 128.57, 128.40, 128.35, 128.25, 127.97, 127.90, 127.81, 127.71, 127.58, 127.53, 127.31, 87.63, 79.51, 79.18, 75.17, 75.08, 73.29, 72.86, 72.69, 64.68, 64.43, 62.65, 62.52, 56.81, 56.55, 47.94, 37.57, 36.71, 33.02, 32.28, 32.03, 31.53, 30.55, 30.16, 29.71, 29.33, 29.08, 26.09, 23.58, 22.97, 22.70, 18.53, 14.23; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{44}H_{56}NO_4$: 662.4209, found 662.4226; IR (neat, NaCl, cm^{-1}): 3089, 3064, 2922, 2855, 1726, 1605, 1586, 1496, 1454, 1361, 1316, 1268, 1207, 1173, 1095, 1028, 903, 820, 734, 697. $[\alpha]_D^{25}$ -14 (*c* = 0.85, $CHCl_3$).



(2*R*,3*R*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)-1-((1*s*,4*R*)-4-hexyl-cyclohexyl)piperidine (2-3). After chromatographic purification, a colorless oil was obtained (54%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.36 - 7.25 (m, 18H), 7.18 (m, 2H), 4.95 (d, *J* = 11.3 Hz, 1H), 4.88 (d, *J* = 10.9 Hz, 1H), 4.82 (d, *J* = 11.3 Hz, 1H), 4.66 (ddd, *J* = 11.6, 11.6, 11.6 Hz, 2H), 4.59 (d, *J* = 12.3 Hz, 1H), 4.51 (d, *J* = 10.9 Hz, 1H), 4.31 (d, *J* = 12.3 Hz, 1H), 3.60 (m, 2H), 3.57 (m, 1H), 3.49 (dd, *J* = 2.1, 10.4 Hz, 1H), 3.43 (t, *J* = 9.0 Hz, 1H), 3.14 (dd, *J* = 4.9, 11.1 Hz, 1H), 2.63 (t, *J* = 11.6 Hz, 1H), 2.42 (d, *J* = 9.5 Hz, 1H), 2.02 (t, *J* = 10.8 Hz, 1H), 1.79 (d, *J* = 12.7 Hz, 1H), 1.64 (dt, *J* = 2.8, 10.3 Hz, 2H), 1.58 (m, 2H), 1.51 (d, *J* = 12.3 Hz, 1H), 1.40 - 1.25 (m, 9H), 1.13 - 1.01 (m, 4H), 0.89 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 139.22, 138.63, 137.70, 128.69, 128.39, 128.35, 128.25, 127.97, 127.90, 127.81, 127.71, 127.58, 127.53, 127.31, 87.63, 79.33, 79.18, 75.17, 75.08, 73.29, 72.69, 72.69, 64.42, 62.65, 56.55, 47.93, 37.58, 37.03, 33.01, 32.03, 31.93, 29.61, 29.33, 27.04, 23.58, 22.97, 22.69, 14.15; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{46}H_{60}NO_4$: 690.4522, found 690.4520; IR (neat, NaCl, cm^{-1}): 3088, 3064, 3030, 2923, 2854, 1726, 1604, 1586, 1497, 1454, 1361, 1315, 1270, 1207, 1173, 1096, 1028, 902, 820, 734, 697. $[\alpha]_D^{25}$ -12 (*c* = 0.85, $CHCl_3$).

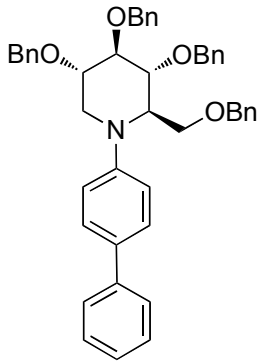


(2*R*,3*R*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)-1-phenylpiperidine (2-4). After chromatographic purification, a yellow oil was obtained (46%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.39 - 7.21 (m, 22H), 7.07 (d, *J* = 8.5 Hz, 2H), 7.02 (t, *J* = 7.4 Hz, 1H), 4.90 (m, 1H), 4.85 (d, *J* = 5.5 Hz, 1H), 4.81 (m, 2H), 4.70 (m, 2H), 4.58 (d, *J* = 11.2 Hz, 1H), 4.41 (d, *J* = 12.0 Hz, 1H), 4.34 (d, *J* = 12.0 Hz, 1H), 4.02 (t, *J* = 6.9 Hz, 1H), 3.87 (m, 1H), 3.75 (t, *J* = 7.7 Hz, 1H), 3.66 (dd, *J* = 2.0, 9.6 Hz, 1H), 3.47 (dd, *J* = 4.8, 11.4 Hz, 1H), 3.41 (dd, *J* = 4.6, 9.8 Hz, 1H), 3.04 (m, 1H), 3.07 (t, *J* = 10.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 150.45, 139.19, 138.85, 138.80, 138.53, 129.5, 128.92, 128.83, 128.78, 128.38, 128.24, 128.19, 128.10, 128.03, 127.96, 127.73, 122.44, 120.66, 85.77, 79.49, 78.11, 74.54, 74.22, 73.53, 72.79, 67.66, 63.12, 52.72; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{40}H_{42}NO_4$: 600.3114, found 600.3113; IR (neat, NaCl, cm^{-1}): 3060, 3027, 2922, 2852, 1721, 1599, 1497, 1453, 1364, 1269, 1207, 1095, 1077, 1027, 749, 735, 696. $[\alpha]_D^{25}$ -2.3 (*c* = 0.47, $CHCl_3$).



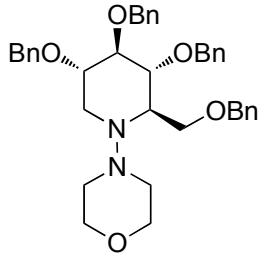
(2*R*,3*R*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)-1-(4-hexylphenyl)-piperidine (2-5). After chromatographic purification, a yellow oil was obtained (44%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.33 - 7.18 (m, 20H),

7.09 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 4.89 (d, *J* = 11.3 Hz, 1H), 4.81 (t, *J* = 12.4 Hz, 2H), 4.57 (d, *J* = 11.3 Hz, 1H), 4.41 (d, *J* = 12.4 Hz, 1H), 4.27 (d, *J* = 12.4 Hz, 1H), 3.98 (t, *J* = 9.1 Hz, 1H), 3.88 (m, 1H), 3.75 (t, *J* = 7.9 Hz, 1H), 3.66 (d, *J* = 11.4 Hz, 1H), 3.45 (dd, *J* = 2.0, 9.6 Hz, 1H), 3.34 (d, *J* = 11.4 Hz, 1H), 3.14 (m, 1H), 2.98 (t, *J* = 11.4 Hz, 1H), 2.57 (t, *J* = 9.1 Hz, 2H), 1.57 (m, 4H), 1.30 (m, 6H), 0.88 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 147.77, 138.92, 138.53, 138.40, 138.08, 137.98, 128.94, 128.45, 128.37, 128.31, 128.23, 128.04, 127.94, 127.90, 127.78, 127.75, 127.61, 127.54, 127.47, 127.44, 127.30, 122.10, 116.30, 86.07, 83.09, 79.61, 78.93, 75.55, 74.55, 74.35, 73.20, 73.07, 72.43, 66.74, 63.92, 58.76, 54.55, 35.32, 35.02, 34.97, 31.75, 31.57, 29.70, 29.05, 22.63, 14.13; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{46}H_{54}NO_4$: 684.4053, found 684.4028; IR (neat, NaCl, cm^{-1}): 3431, 3351, 3217, 3017, 2956, 2926, 2854, 1875, 1717, 1624, 1517, 1466, 1378, 1273, 1179, 1125, 1071, 1028, 823, 733, 698, 551; $[\alpha]_D^{25}$ 1.2 (*c* = 1.1, $CHCl_3$).



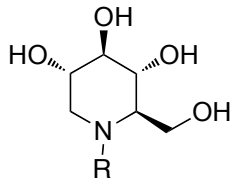
(2*R*,3*R*,4*R*,5*S*)-1-([1,1'-Biphenyl]-4-yl)-3,4,5-tris(benzyloxy)-2-((benzyloxy)-methyl)piperidine (2-6). After chromatographic purification, a yellow oil was obtained (36%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.32 - 7.21 (m, 27H), 7.04

(d, *J* = 6.8 Hz, 2H), 4.77 (m, 2H), 4.72 (t, *J* = 5.0 Hz, 1H), 4.68 (m, 2H), 4.56 (d, *J* = 11.4 Hz, 1H), 4.41 (d, *J* = 12.1 Hz, 1H), 4.36 (d, *J* = 11.9 Hz, 1H), 4.01 (t, *J* = 6.2 Hz, 1H), 3.86 (m, 1H), 3.75 (t, *J* = 6.6 Hz, 1H), 3.66 (dd, *J* = 2.3, 9.2 Hz, 1H), 3.51 (d, *J* = 5.2 Hz, 1H), 3.48 (m, 2H), 3.15 (t, *J* = 9.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 128.69, 128.40, 128.33, 128.29, 127.91, 127.74, 127.64, 127.53, 126.60, 118.89, 84.83, 76.55, 73.83, 73.40, 73.15, 72.30, 70.66, 65.19, 62.00, 50.76; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{46}H_{46}NO_4$: 676.3426, found 676.3428.



4-((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)morpholine (2-7). After chromatographic purification, a viscous yellow oil was obtained (65%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.35 - 7.18 (m, 20H), 4.96 (d, *J* = 11.3 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.83 (d, *J* = 10.8 Hz, 1H), 4.70 (ddd, *J* = 11.6, 11.6, 11.6 Hz, 2H), 4.59 (m, 1H), 4.52 (ddd, *J* = 12.4, 12.4, 12.4 Hz, 2H), 4.07 (d, *J* = 9.4 Hz, 1H), 3.74 (t, *J* = 9.3 Hz, 1H), 3.62 (m, 6H), 3.42 (t, *J* = 9.0 Hz, 1H), 3.29 (dd, *J* = 4.6, 10.0 Hz, 1H), 2.68 (s, 2H), 2.57 (m, 1H), 2.54 (m, 2H), 2.21 (t, *J* = 10.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 138.99, 138.52, 138.35, 128.46, 128.36, 128.30, 127.99, 127.85, 127.77, 127.69, 127.60, 127.54, 127.42, 87.00, 77.78, 75.35, 75.20, 73.30, 67.13, 65.34, 64.12, 46.60;

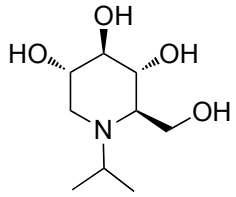
ESI-HRMS: calc'd *m/e* for $(M+H^+)]$ $C_{38}H_{45}N_2O_5$: 609.3328, found 609.3307; IR (neat, NaCl, cm^{-1}): 3063, 3030, 2950, 2912, 2853, 1721, 1604, 1496, 1453, 1361, 1318, 1264, 1207, 1111, 1070, 1028, 919, 866, 735, 697; $[\alpha]_D^{25}$ -5.8 (*c* = 0.91, $CHCl_3$).



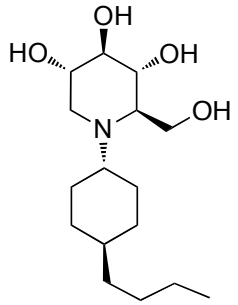
General Procedure for the Synthesis of (2*R*,3*R*,4*R*,5*S*)-2-(Hydroxymethyl)-1-alkylpiperidine-3,4,5-triol (3). Method A: The above tetrabenzyl derivatives were dissolved in MeOH (25 mL/mmol). To the solution was added palladium chloride (0.68 equiv). The reaction was shaken under hydrogen at 1 atmosphere until uptake of hydrogen ceased. The reaction was then carefully filtered through a pressed pad of Celite and evaporated under vacuo. The resultant residue was subsequently dissolved in a minimum amount of 30% aqueous MeOH and loaded onto a Dowex 50W × 8 (200 mesh) ion exchange column. The column was first eluted with a large amount of H_2O, followed by elution with 1M ammonium hydroxide. The fractions containing the product were combined, and the H_2O was evaporated. The resultant residue was purified by flash column chromatography on silica gel, using 10% MeOH/ CH_2Cl_2 + 1% NH_4OH (v/v) as the eluent.

Method B[128]: A mixture of the above tetrabenzyl derivatives, ammonium formate (0.3 g per mmol of *O*-benzyl group) and 10% palladium on carbon (0.35

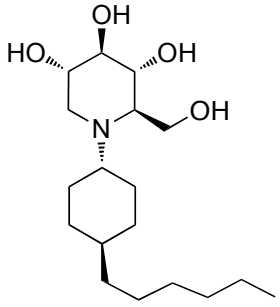
g per mmol of *O*-benzyl group) was refluxed in MeOH in a sealed reaction vessel. The reaction was monitored by thin layer chromatography, and the catalyst was carefully filtered off by passing the reaction mixture through a Celite pad upon completion of the cleavage of the benzyl ether. The solvent was subsequently removed under vacuum. The neutral residue was purified by flash column chromatography on silica gel, using 10% MeOH/ CH_2Cl_2 + 1% NH_4OH (v/v) as the eluent.



(2*R*,3*R*,4*R*,5*S*)-2-(Hydroxymethyl)-1-isopropylpiperidine-3,4,5-triol (3-1). Method B: After chromatographic purification, a colorless oil was obtained (73%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 3.91 (d, *J* = 2.5 Hz, 2H), 3.52 (m, 1H), 3.47 (m, 1H), 3.40 (t, *J* = 9.3 Hz, 1H), 3.15 (t, *J* = 9.0 Hz, 1H), 3.03 (dd, *J* = 4.8, 11.1 Hz, 1H), 2.34 (dd, *J* = 9.6 Hz, 1H), 2.11 (t, *J* = 10.9 Hz, 1H), 1.19 (d, *J* = 6.7 Hz, 3H), 0.98 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 80.7, 79.3, 72.0, 71.0, 66.2, 58.5, 21.4, 13.1; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_9H_{20}NO_4$: 206.1392, found 206.1405; $[\alpha]_D^{25}$ -11 (*c* = 0.91, MeOH).

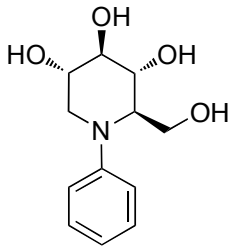


(2*R*,3*R*,4*R*,5*S*)-1-((1*S*,4*R*)-4-Butylcyclohexyl)-2-(hydroxymethyl)piperidine-3,4,5-triol (3-2). Method A. After chromatographic purification, a colorless oil was obtained (75%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 3.95 (m, 1H), 3.82 (d, *J* = 2.5 Hz, 1H), 3.51 (m, 2H), 3.31 (m, 1H), 3.11 (m, 1H), 2.88 (m, 1H), 2.40 (m, 1H), 2.18 (m, 1H), 1.83 (m, 2H), 1.76 (m, 6H), 1.06 (m, 7H), 0.86 (t, *J* = 10.2 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 79.4, 70.5, 69.8, 63.7, 57.2, 56.3, 49.7, 37.7, 36.7, 33.1, 32.3, 29.4, 23.0, 14.0; ESI-HRMS: calc'd *m/e* for [M+H^{+}] $C_{16}H_{32}NO_4$: 302.2331, found 302.2306; $[\alpha]_D^{25}$ -46 (*c* = 0.99, MeOH).

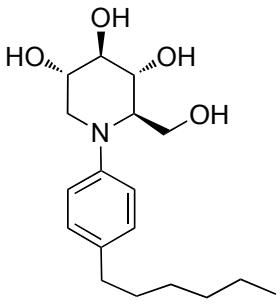


(2*R*,3*R*,4*R*,5*S*)-1-((1*S*,4*R*)-4-Hexylcyclohexyl)-2-(hydroxymethyl)piperidine-3,4,5-triol (3-3). Method A: After chromatographic purification, a colorless oil was obtained (60%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 3.96 (d, *J* = 8.8 Hz, 1H), 3.80 (d, *J* = 8.6 Hz, 1H), 3.65 (m, 2H), 3.33 (m, 1H), 3.12 (m, 1H), 2.89 (m, 1H), 2.45 (d, *J* = 4.7 Hz, 1H), 2.21 (m, 1H), 1.82 (m, 3H), 1.71 (m, 2H), 1.51 (d, *J*

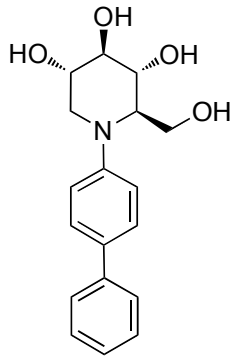
= 7.9 Hz, 1H), 1.25 (m, 8H), 1.16 (m, 4H), 1.01 (d, *J* = 9.6 Hz, 1H), 0.89 (t, *J* = 10.4 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 79.1, 70.4, 69.6, 63.7, 57.7, 57.0, 49.6, 37.7, 37.0, 33.0, 31.9, 31.0, 29.7, 27.2, 22.7, 14.1; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{18}H_{36}NO_4$: 330.2645, found 302.2638; $[\alpha]_D^{25}$ -35 (*c* = 0.74, MeOH).



(2*R*,3*R*,4*R*,5*S*)-2-(Hydroxymethyl)-1-phenylpiperidine-3,4,5-triol (3-4). Method A: After chromatographic purification, a colorless oil was obtained (78%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 7.32 (d, *J* = 6.7 Hz, 2H), 7.22 (d, *J* = 6.7 Hz, 2H), 7.09 (m, 1H), 3.75 (m, 1H), 3.72 (d, *J* = 4.5 Hz, 2H), 3.53 (d, *J* = 8.9 Hz, 1H), 3.45 (t, *J* = 8.4 Hz, 1H), 3.25 (d, *J* = 11.2 Hz, 1H), 3.02 (m, 1H), 2.91 (t, *J* = 11.6 Hz, 1H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 151.8, 130.2, 125.1, 124.4, 78.8, 72.6, 71.4, 66.4, 62.8, 60.4, 59.1; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{12}H_{18}NO_4$: 240.1236, found 240.1233; $[\alpha]_D^{25}$ 6.3 (*c* = 0.98, MeOH).

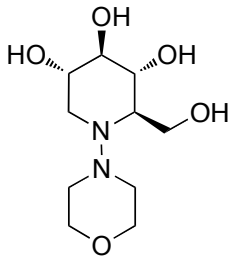


(2*R*,3*R*,4*R*,5*S*)-1-(4-Hexylphenyl)-2-(hydroxymethyl)piperidine-3,4,5-triol (3-5). Method A: After chromatographic purification, a colorless oil was obtained (37%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 7.05 (m, 4H), 3.85 (m, 2H), 3.71 (d, *J* = 11.0 Hz, 1H), 3.58 (t, *J* = 9.1 Hz, 1H), 3.44 (d, *J* = 10.5 Hz, 1H), 3.21 (d, *J* = 7.1 Hz, 1H), 2.85 (d, *J* = 8.0 Hz, 1H), 2.78 (t, *J* = 9.5 Hz, 1H), 2.52 (t, *J* = 7.8 Hz, 1H), 1.55 (m, 2H), 1.29 (m, 6H), 1.21 (t, *J* = 7.0 Hz, 1H), 0.88 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 147.1, 140.3, 129.3, 124.7, 78.7, 71.2, 69.9, 65.9, 65.1, 60.1, 59.5, 35.5, 31.7, 31.4, 29.1, 22.6, 15.3, 14.1; ESI-HRMS: calc'd *m/e* for [M+H^{+}] $C_{18}H_{30}NO_4$: 324.2175, found 324.2168; $[\alpha]_D^{25}$ 1.5 (*c* = 0.20, MeOH).



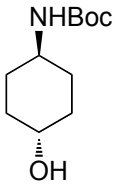
(2*R*,3*R*,4*R*,5*S*)-1-([1,1'-Biphenyl]-4-yl)-2-(hydroxymethyl)piperidine-3,4,5-triol (3-6). Method A: After chromatographic purification, a yellow oil was obtained (15%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 7.60 - 7.09 (m, 9H), 3.79 (m, 1H), 3.74 (m, 1H), 3.66 (m, 1H), 3.49 (t, *J* = 6.2 Hz, 1H), 3.18 (m, 1H), 3.04 (dd, *J* = 2.8, 9.5 Hz, 1H), 1.83 (t, *J* = 7.0 Hz, 1H), 1.42 (d, *J* = 12.2 Hz, 1H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 128.4, 127.2, 126.4, 126.2, 122.2, 76.7, 71.1, 70.1, 64.8, 59.1, 56.2; ESI-HRMS: calc'd *m/e* for [M+H^{+}] $C_{18}H_{22}NO_4$: 316.1549, found

316.1544; $[\alpha]_D^{25}$ 17 (*c* = 0.25, MeOH).



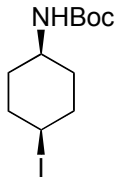
(2*R*,3*R*,4*R*,5*S*)-2-(Hydroxymethyl)-1-morpholinopiperidine-3,4,5-triol (3-7)

Method B: After chromatographic purification, a colorless oil was obtained (45%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 4.11 (dd, *J* = 2.4, 11.2 Hz, 1H), 3.78 (dd, *J* = 5.6, 13.6 Hz, 2H), 3.69 (m, 2H), 3.42 (m, 1H), 3.37 (m, 1H), 3.21 (m, 1H), 3.15 (d, *J* = 8.6 Hz, 1H), 3.11 (t, *J* = 8.5 Hz, 1H), 2.91 (m, 2H), 2.70 (m, 2H), 2.60 (m, 1H), 2.32 (t, *J* = 10.2 Hz, 1H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 80.3, 79.6, 72.1, 70.7, 68.9, 68.2, 65.6, 63.8; ESI-HRMS: calc'd *m/e* for $[M+Na^+]$ $C_{10}H_{20}N_2NaO_5$: 271.1270, found 271.1267; $[\alpha]_D^{25}$ -23 (*c* = 0.98, MeOH).



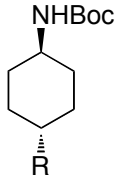
***tert*-Butyl (*trans*-4-Hydroxycyclohexyl)carbamate (4).**[38] The HCl salt of the starting primary amine (2.5 g, 16.6 mmol) was dissolved in H_2O and dioxane (64 mL, 1:3, v/v). 1N NaOH (17 mL, 17.0 mmol) was then added to the solution to neutralize the HCl salt. After the addition was completed, the reaction was cooled to 0 °C, and Boc_2O (4.0 g, 18.3 mmol) was added and the mixture was

stirred in the open air for 2 h. The reaction was quenched with 1N potassium bisulfate solution (20 mL) and then extracted with chloroform (100 mL × 2). The organic layers were combined, dried over magnesium sulfate and subjected to rotary evaporation. To the resultant crude white solid was added with a mixture of chloroform and hexanes (80 mL, 1:2, v/v). The suspension was heated until fully dissolved, and was then stored in a 4 °C fridge. The pure compound precipitated out overnight as a colorless feather-like solid (55%, mp: 169 - 170 °C). The observed spectroscopic data were in agreement with the data in the literature. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.38 - 4.36 (br s, 1H), 3.64 (m, 1H), 3.45 - 3.43 (br s, 1H), 2.01 (d, *J* = 15.6 Hz, 4H), 1.47 (s, 9H), 1.39 (m, 3H), 1.19 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 155.3, 79.3, 69.8, 49.0, 34.0, 31.1, 28.4; IR (neat, NaCl, cm^{-1}): 3338, 2938, 2892, 1683, 1529, 1453, 1365, 1318, 1273, 1267, 1248, 1178, 1070, 952, 892.



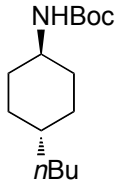
***tert*-Butyl (*cis*-4-Iodocyclohexyl)carbamate (5).** Intermediate **5** was prepared according to a literature procedure[129] using the above material *tert*-butyl (*trans*-4-hydroxycyclohexyl)carbamate (**4**) as the starting material. Triphenylphosphine (548.3 mg, 2.1 mmol) and imidazole (359.3 mg, 5.3 mmol) were successively dissolved in toluene (15 mL). The resultant mixture was cooled to 0 °C, and iodine (514 mg, 2.0 mmol) was added in portions over 30 min. The solution was

then warmed to room temperature and stirred for 10 min. A solution of **4** (223 mg, 1.04 mmol) in toluene (5 mL) was cannuled into the reaction; the reaction was then heated to 60 °C and stirred for 1 h. After cooling, the reaction was quenched with H_2O (25 mL), and the aqueous layer was extracted with EtOAc (25 mL × 2). The organic layers were combined, washed with 10% sodium thiosulfate, dried over magnesium sulfate and concentrated *in vacuo*. The crude compound was purified by flash column chromatography on silica gel, using 10% EtOAc:hexanes (v/v) as the eluent to give a white solid (77%, mp: 95 - 96 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.66 (s, 1H), 4.57 (s, 1H), 3.55 (s, 1H), 2.08 (m, 2H), 1.82 (m, 2H), 1.66 (m, 4H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 155.2, 79.3, 48.1, 35.4, 30.1, 28.6; IR (neat, NaCl, cm^{-1}): 3331, 2975, 2938, 1697, 1522, 1455, 1440, 1390, 1365, 1348, 1320, 1249, 1227, 1166, 1066, 1043, 1014, 987, 880, 852, 782.

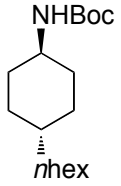


General Procedure for the Synthesis of *tert*-Butyl (*trans*-4-Alkylcyclohexyl)carbamates 6. In a flame-dried flask was placed copper cyanide (CuCN). The vessel was flushed with argon and then evacuated under high vacuum. The process was repeated three times, and the dry CuCN was kept under argon. Dry THF (1 mL/mmol CuCN) was added via syringe and the slurry was cooled to -78 °C with a dry ice-acetone bath. To the slowly stirring

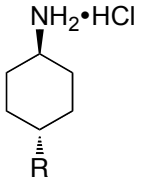
suspension was added the organ lithium reagents (2 equiv relative to CuCN) dropwise. The heterogeneous mixture was allowed to warm gradually to 0 °C until a clear solution was formed. Next, the solution was re-cooled to -78 °C. A solution of the iodo species **5** in THF was then added to the solution. The reaction was stirred at -78 °C until all the starting material was consumed. The reaction was then quenched with a solution of 10% concentrated NH_4OH in saturated NH_4Cl, and was allowed to stir at room temperature for 30 min. The aqueous phase was then extracted with EtOAc (15 mL × 2). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 10% EtOAc:hexanes as the eluent.



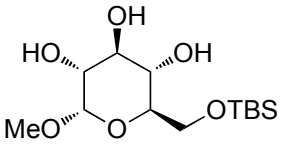
***tert*-Butyl (*trans*-4-Butylcyclohexyl)carbamate (6-1).** After chromatographic purification, a white solid was obtained (82%, mp: 98 - 99 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.43 (s, 1H), 3.34 (s, 1H), 1.97 (d, *J* = 12.1 Hz, 2H), 1.76 (d, *J* = 12.1 Hz, 2H), 1.44 (s, 9H), 1.26 - 0.93 (m, 11H), 0.83 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 154.2, 77.9, 49.0, 38.2, 36.0, 34.7, 32.6, 29.9, 28.7, 21.7, 14.7; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{15}H_{29}NNaO_2$: 278.2096, found 278.2070; IR (neat, NaCl, cm^{-1}): 3364, 2971, 2930, 2848, 1682, 1526, 1445, 1390, 1365, 1322, 1289, 1253, 1235, 1178, 1092, 1045, 882.



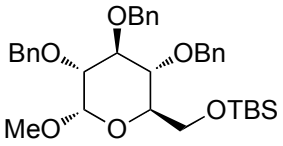
***tert*-Butyl (*trans*-4-Hexylcyclohexyl)carbamate (6-2).** After chromatographic purification, a white solid was obtained (78%, mp: 77 - 78 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.44 (s, 1H), 3.35 (s, 1H), 1.96 (d, *J* = 12.2 Hz, 2H), 1.74 (d, *J* = 12.2 Hz, 2H), 1.43 (s, 9H), 1.24 (m, 8H), 1.15 - 0.92 (m, 7H), 0.86 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 154.0, 77.7, 50.0, 48.8, 38.1, 35.7, 32.4, 30.6, 28.8, 27.2, 26.0, 21.7, 14.7; ESI-HRMS: calc'd *m/e* for [M+Na^+] $C_{17}H_{33}NNaO_2$: 306.2409, found 306.2417; IR (neat, NaCl, cm^{-1}): 3371, 2926, 2850, 1683, 1522, 1451, 1389, 1366, 1321, 1270, 1247, 1239, 1178, 1092, 1044, 1029, 901, 872, 801.



General Procedure for the Synthesis of *trans*-4-Alkylcyclohexanamine Hydrochlorides 7. The above carbamates were placed in a flame-dried flask and cooled to 0 °C. To the solid material, 4M HCl in dioxane (5 equiv relative to **6**) was added. The reaction was slowly stirred at 0 °C until all the start material was consumed. The solvent was then removed under high vacuum, and the crude solid product was directly used in the reductive alkylation reaction.

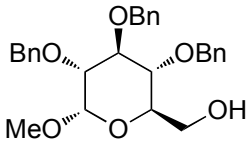


Methyl 6-*O*-tert-Butyldimethylsilyl-α-D-pyranoside (8). Methyl-alpha-D-glucopyranoside (5.1 g, 26.3 mmol) was dissolved in anhydrous DMF (60 mL) and cooled to 0 °C. Imidazole (4.4 g, 64.6 mmol) and *tert*-butyldimethylsilyl chloride (4.7 g, 31.2 mmol) were added to the solution in sequence. The mixture was allowed to stir at room temperature overnight. Upon the consumption of starting material, the solvent was removed under high vacuum and the residue was dissolved in EtOAc (400 mL). The solution was washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel, using 100% EtOAc as the eluent. After chromatographic purification, an amorphous solid was obtained (90%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.75 (d, *J* = 3.9 Hz, 1H), 3.89 (dd, *J* = 4.9, 10.6 Hz, 1H), 3.82 (dd, *J* = 5.2, 10.6 Hz, 1H), 3.74 (t, *J* = 9.1 Hz, 1H), 3.61 (dd, *J* = 5.0, 9.7 Hz, 1H), 3.53 (t, *J* = 9.0 Hz, 1H), 3.51 (m, 1H), 3.42 (s, 3H), 3.14 (s, 1H), 2.82 (s, 1H), 2.19 (d, *J* = 7.6 Hz, 1H), 0.91 (s, 9H), 0.10 (s, 6H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 99.1, 74.8, 72.5, 72.2, 70.3, 64.3, 55.3, 25.9, 18.3, -5.5; ESI-HRMS: calc'd *m/e* for [M+Na^+] $C_{13}H_{28}NaO_6Si$: 309.1733, found 309.2209; IR (neat, NaCl, cm^{-1}): 3474, 2954, 2929, 2858, 1655, 1472, 1464, 1361, 1253, 1194, 1152, 1112, 1045, 1002, 903, 854, 837, 777, 749; $[\alpha]_D^{25}$ 98 (*c* = 1.0, $CHCl_3$).



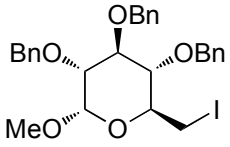
Methyl-2,3,4-tri-*O*-benzyl-6-*O*-tert-butyldimethylsilyl-α-D-pyranoside (9). Methyl 6-*O*-tert-butyldimethylsilyl-α-D-pyranoside (**8**) (7.0 g, 22.7 mmol) was dissolved in anhydrous DMF (100 mL) and cooled to 0 °C. Sodium hydride (3.3 g, 82.5 mmol) was added to the solution. After the evolution of H_2 ceased, benzyl bromide (12.2 mL, 102.6 mmol) was added to the reaction. The mixture was stirred under an atmosphere of nitrogen overnight. Upon the consumption of the starting material, the reaction was quenched with MeOH (10 mL), then poured into water (500 mL) and extracted with EtOAc (5 × 100 mL). The combined organic layers were washed with water (2 × 200 mL) and brine (2 × 200 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel, using 10% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (85%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.32 - 7.21 (m, 15H), 4.93 (d, *J* = 10.8 Hz, 1H), 4.84 (d, *J* = 10.9 Hz, 1H), 4.78 (d, *J* = 10.9 Hz, 1H), 4.76 (d, *J* = 12.1 Hz, 1H), 4.64 (d, *J* = 12.1 Hz, 1H), 4.60 (d, *J* = 11.0 Hz, 1H), 4.57 (d, *J* = 3.6 Hz, 1H), 3.96 (t, *J* = 9.3 Hz, 1H), 3.75 (d, *J* = 3.2 Hz, 1H), 3.58 (dt, *J* = 3.7, 10.0 Hz, 1H), 3.50 (d, *J* = 9.1 Hz, 1H), 3.48 (d, *J* = 3.6 Hz, 1H), 3.46 (d, *J* = 3.6 Hz, 1H), 3.33 (s, 3H), 0.85 (s, 9H), 0.00 (d, *J* = 2.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 138.82, 138.53, 138.29, 128.51, 128.42, 128.40, 128.29, 128.10, 128.06, 127.83, 127.67, 127.61, 126.12, 97.90, 82.18, 80.27, 77.78, 75.85, 74.99, 73.35, 71.52, 62.29, 54.89, 25.93, -5.16, -5.37; ESI-HRMS: calc'd

m/e for [M+Na$^+$] $C_{34}H_{46}NaO_6Si$: 601.2961, found 601.2932; IR (neat, NaCl, cm^{-1}): 3065, 3031, 2928, 2856, 1606, 1497, 1455, 1361, 1252, 1201, 1193, 1160, 1136, 1092, 1072, 910, 836, 778, 735, 697; $[\alpha]_D^{25}$ 130 (*c* = 0.89, $CHCl_3$).



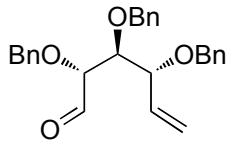
Methyl-2,3,4-tri-*O*-benzyl-α-D-pyranoside (10). Methyl-2,3,4-tri-*O*-benzyl-6-*O*-tert-butyldimethylsilyl-α-D-pyranoside (**9**) (11.5 g, 19.9 mmol) was dissolved in acetonitrile (120 mL). H_2O (25 mL) was added, and the pH of the solution adjusted to pH = 3 by the addition of *p*-toluenesulphonic acid. The reaction mixture was stirred at room temperature overnight. Upon the consumption of the starting material, EtOAc (300 mL) was added to the reaction mixture. The mixture was washed with saturated aqueous sodium bicarbonate (2 × 150 mL) and brine (150 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel, using 30% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (65%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 - 7.28 (m, 15H), 4.99 (d, *J* = 10.9 Hz, 1H), 4.88 (d, *J* = 11.1 Hz, 1H), 4.84 (d, *J* = 10.9 Hz, 1H), 4.80 (d, *J* = 12.1 Hz, 1H), 4.65 (t, *J* = 12.2 Hz, 2H), 4.57 (d, *J* = 3.6 Hz, 1H), 4.00 (t, *J* = 9.3 Hz, 1H), 3.77 (m, 1H), 3.69 (m, 1H), 3.65 (m, 1H), 3.53 (d, *J* = 9.2 Hz, 1H), 3.50 (dd, *J* = 3.6, 6.1 Hz, 1H), 3.37 (s, 3H), 1.60 (dd, *J* = 5.3, 7.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 138.77, 138.16, 128.49, 128.42, 128.14, 128.05, 127.98, 127.95, 127.89,

127.63, 98.21, 82.98, 80.02, 77.44, 75.76, 75.05, 73.45, 70.67, 61.93, 55.20; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{28}H_{32}NaO_6$: 487.2097, found 487.2104; IR (neat, NaCl, cm^{-1}): 3486, 3063, 3034, 2921, 1732, 1606, 1497, 1454, 1360, 1260, 1192, 1076, 1072, 1068, 911, 802, 737, 697; $[\alpha]_D^{25}$ 23 (*c* = 0.99, $CHCl_3$).



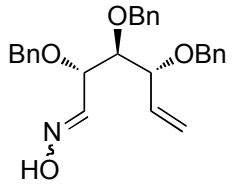
(2S,3S,4S,5R,6S*)*-3,4,5-Tris(benzyloxy)-2-(iodomethyl)-6-methoxytetrahydro-2H-pyran (11). Triphenylphosphine (5.1 g, 19.4 mmol) and imidazole (1.8 g, 26.4 mmol) were successively dissolved in THF (150 mL). The resultant mixture was cooled to 0 °C, and iodine (4.9 g, 19.3 mmol) was added in portions over 30 min. A solution of **10** (6.0 g, 12.9 mmol) in THF (50 mL) was cannuled into the reaction flask. The reaction was then heated to 66 °C and stirred at that temperature for 1 h. The reaction was then cooled to room temperature and quenched with H_2O (25 mL); the aqueous layer was extracted with EtOAc (250 mL × 2). The organic layers were combined, washed with 10% sodium thiosulfate, dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 10% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a light yellow oil was obtained (97%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 - 7.27 (m, 15H), 4.99 (d, *J* = 10.8 Hz, 1H), 4.94 (d, *J* = 11.0 Hz, 1H), 4.81 (d, *J* = 2.0 Hz, 1H), 4.79 (d, *J* = 3.3 Hz, 1H), 4.67 (t, *J* = 11.0 Hz, 2H), 4.61 (d, *J* = 3.6 Hz, 1H), 4.01 (t, *J* = 9.0 Hz, 1H), 3.54 (dd, *J* = 3.6 Hz, 1H), 3.46 (m, 2H), 3.42 (s, 3H), 3.34

(t, *J* = 9.0 Hz, 1H), 3.29 (dd, *J* = 6.7, 11.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 138.56, 138.05, 138.02, 128.53, 128.51, 128.45, 128.10, 128.01, 127.95, 127.71, 98.13, 81.58, 81.51, 80.10, 75.80, 75.37, 73.46, 69.31, 55.52, 7.65; ESI-HRMS: calc'd *m/e* for $[M+Na^+]$ $C_{28}H_{31}INaO_5$: 597.1114, found 597.1162; IR (neat, NaCl, cm^{-1}): 3062, 3030, 2909, 1496, 1454, 1359, 1260, 1196, 1120, 1102, 1088, 1048, 1028, 736, 697; $[\alpha]_D^{25}$ 32 (*c* = 1.1, $CHCl_3$).



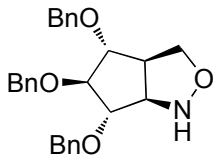
(2*R*,3*S*,4*R*)-2,3,4-Tris(benzyloxy)hex-5-enal (12). Compound **11** (4.6 g, 8.0 mmol) was dissolved in a mixture of THF/H_2O (9:1, 200 mL) and then activated zinc (5.2 g, 79.5 mmol) was added. The flask was placed in an ultrasonic cleaner (Fisher, FS20H) and sonicated overnight. The reaction progress was monitored by mass-spectroscopy due to the fact that the Rf value of the product is identical to that of the starting material on TLC. Upon the consumption of the starting material, the reaction was diluted with EtOAc (50 mL), and the aqueous layer was extracted with EtOAc (50 mL × 2). The organic layers were combined, washed with sodium bicarbonate (20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography on silica gel, using 10% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (94%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 9.63, 9.57 (rotamer, 1H), 7.29 - 7.21 (m, 15H), 5.76 (m, 1H), 5.29 (dt, *J* = 1.0, 4.3 Hz, 1H), 5.20 (dd, *J* = 1.0, 7.0 Hz, 1H), 4.64 (dd, *J*

= 2.2, 10.0 Hz, 1H), 4.60 (t, *J* = 13.1 Hz, 1H), 4.54 (m, 1H), 4.50 (m, 1H), 4.44 (dd, *J* = 8.0, 19.8 Hz, 1H), 4.30 (dd, *J* = 3.3, 11.9 Hz, 1H), 4.04 (m, 1H), 3.91 (t, *J* = 6.8 Hz, 1H), 3.78 (m, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 201.24 (201.60, 201.98), 140.93, 138.35, 138.23, 138.09, 138.06, 137.84, 137.70, 137.22, 134.80, 134.40, 128.56, 128.50, 128.45, 128.40, 128.38, 128.34, 128.30, 128.25, 128.17, 128.12, 128.02, 127.98, 127.93, 127.91, 127.79, 127.68, 127.64, 127.60, 126.98, 119.44, 82.40, 80.26, 79.95, 75.94, 74.50, 73.19, 70.85; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{27}H_{28}NaO_4$: 439.1885, found 439.1954; IR (neat, NaCl, cm^{-1}): 3064, 3031, 2866, 1727, 1497, 1454, 1393, 1352, 1208, 1071, 1027, 932, 735, 697; $[\alpha]_D^{25}$ 4.5 (*c* = 0.85, $CHCl_3$).



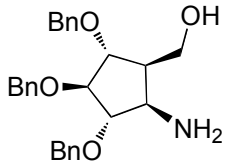
(2*S*,3*S*,4*R*)-2,3,4-Tris(benzyloxy)hex-5-enal Oxime (13). Aldehyde **12** (3.8 g, 9.1 mmol) was dissolved in MeOH (40 mL). To the stirring solution, hydroxylamine hydrochloride (2.5 g, 36.0 mmol) was added. The suspension was neutralized with sodium bicarbonate (4.3 g, 40.6 mmol). The solution was then stirred under refluxing conditions for 6 h. Upon the consumption of the starting material, the solvent was removed *in vacuo*. The residue was dissolved in EtOAc, washed with 10% HCl solution, saturated sodium bicarbonate and brine in sequence and dried over $MgSO_4$. The solvent was removed to yield an oily residue which was purified by flash column chromatography on silica gel,

using 25% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a mixture of inseparable *E/Z* isomers as a light green oil was obtained (85%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm, major): δ 7.46 (d, *J* = 7.8 Hz, 1H), 7.31 (m, 15H), 5.76 (m, 1H), 5.24 (m, 1H), 5.20 (d, *J* = 5.2 Hz, 1H), 4.84 (m, 1H), 4.80 (d, *J* = 11.5 Hz, 1H), 4.70 (d, *J* = 11.4 Hz, 1H), 4.60 (d, *J* = 3.7 Hz, 1H), 4.57 (d, *J* = 3.7 Hz, 1H), 4.38 (d, *J* = 9.2 Hz, 1H), 4.35 (d, *J* = 9.3 Hz, 1H), 4.15 (m, 1H), 3.58 (t, *J* = 4.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 150.5, 140.4, 138.6, 137.6, 135.1, 128.45, 128.43, 128.27, 128.32, 128.25, 127.96, 127.74, 127.67, 127.47, 121.3, 120.9, 82.5, 81.1, 76.4, 75.1, 71.2, 70.8; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{27}H_{29}NNaO_4$: 454.1994, found 454.2014; IR (neat, NaCl, cm^{-1}): 3331, 3088, 3064, 3031, 2870, 1642, 1606, 1497, 1454, 1422, 1394, 1359, 1208, 1070, 1028, 996, 933, 818, 736, 698;



(1*R*,2*R*,3*R*,4*S*,5*S*)-4,5,6-Tribenzyloxyhexahydro-1*H*-cyclopent[*c*]isoxazole (14). A stirring solution of oxime **13** (970.5 mg, 2.2 mmol) in dry toluene (35 mL) was heated at reflux for 15 h under nitrogen. Upon the consumption of the starting material, the reaction was cooled; and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 40% EtOAc/hexanes as the eluent. After chromatographic purification, a yellow oil was obtained (70%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.38 - 7.28 (m, 15 H), 4.85 (d, *J* = 11.8 Hz, 1H), 4.79 (d, *J* = 11.9 Hz, 2H), 4.71 (d, *J* = 5.9

Hz, 1H), 4.69 (d, *J* = 6.2 Hz, 1H), 4.58 (d, *J* = 11.8 Hz, 1H), 3.94 (t, *J* = 8.4 Hz, 1H), 3.87 (t, *J* = 5.8 Hz, 1H), 3.85 (m, 1H), 3.83 (t, *J* = 6.6 Hz, 1H), 3.68 (t, *J* = 7.4 Hz, 1H), 3.46 (t, *J* = 6.9 Hz, 1H), 2.91 (dd, *J* = 5.5, 7.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 138.52, 138.25, 138.04, 128.50, 128.36, 128.33, 127.86, 127.76, 127.75, 127.69, 127.60, 127.53, 86.0, 85.6, 84.7, 76.0, 72.7, 72.4, 72.3, 66.3, 49.6; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{27}H_{29}NNaO_4$: 454.1994, found 454.1996; IR (neat, NaCl, cm^{-1}): 3435, 3031, 2922, 2863, 1742, 1724, 1497, 1454, 1364, 1208, 1094, 1072, 736, 697; $[\alpha]_D^{25}$ 25 (*c* = 0.54, $CHCl_3$).

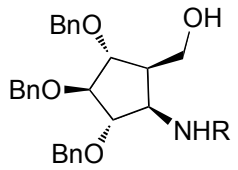


((1*R*,2*R*,3*S*,4*S*,5*R*)-2,3,4-Tris(benzyloxy)-5-amino-cyclopentyl)methanol(15).

To a stirring solution of **14** (675.0 mg, 1.6 mmol) in 85% acetic acid in H_2O (25 mL), active zinc dust (510.0 mg, 8.0 mmol) was added. The reaction was then stirred at 55 °C for 2 h. Upon the consumption of the starting material, the mixture was cooled to room temperature and the zinc dust was filtered off. The filtrate was diluted with H_2O (25 mL) and basified with 1M NaOH. The resultant solution was extracted with CH_2Cl_2 (3 x 25 mL); the organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 10% MeOH/CH_2Cl_2 + 1% NH_4OH as the eluent. After chromatographic purification, a colorless oil was obtained (87%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.30 - 7.28 (m, 15 H),

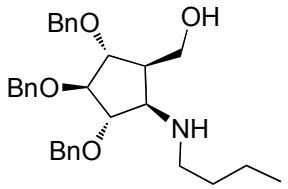
5.56 - 5.23 (br s, 3H), 4.68 (d, *J* = 12.0 Hz, 1H), 4.64 (d, *J* = 11.1 Hz, 1H), 4.54 (m, 2H), 4.53 (d, *J* = 11.6 Hz, 1H), 4.48 (d, *J* = 11.6 Hz, 1H), 4.02 (t, *J* = 5.7 Hz, 1H), 3.92 (t, *J* = 4.4 Hz, 1H), 3.87 (t, *J* = 5.0 Hz, 1H), 3.82 (m, 1H), 3.69 (t, *J* = 6.7 Hz, 1H), 2.46 (m, 1H), 2.02 (m, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 137.88, 127.84, 137.63, 128.44, 127.93, 127.85, 127.81, 87.3, 85.3, 82.7, 72.4, 72.1, 71.9, 60.7, 55.5, 45.0; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{27}H_{32}NO_4$: 434.2326, found 434.2295; IR (neat, NaCl, cm^{-1}): 3308, 3063, 3030, 2927, 2871, 1496, 1454, 1363, 1207, 1091, 1071, 1028, 735, 697.

To prepare the HCl salt of the hydroxycyclopentamine, pure **15** was dissolved in ether and 2M HCl in ether was added at 0 °C. A white solid precipitated out instantly, and the solvent was removed *in vacuo* to afford the HCl salt for the next step.

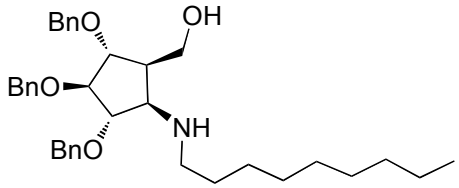


General Procedure for the Synthesis of ((1*R*,2*R*,3*S*,4*S*,5*R*)-2,3,4-Tris(benzyloxy)-5-(alkylamino)cyclopentyl)methanols 16. To a suspension of the HCl salt of the above amine **15**, an equal molar amount of triethylamine was added, followed by the addition with the corresponding aldehyde (0.95 equiv). After 10 min, sodium triacetoxyborohydride (2.0 equiv) was added to the mixture, and the reaction was stirred at room temperature for 2 h. Upon the consumption of the starting material, the reaction was quenched with a saturated sodium

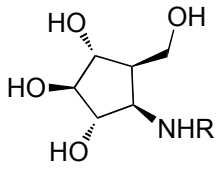
bicarbonate solution and extracted with CH_2Cl_2 (2 x 20 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 5% MeOH/CH_2Cl_2 + 1% NH_4OH as the eluent.



((1*R*,2*R*,3*S*,4*S*,5*R*)-2,3,4-Tris(benzyloxy)-5-(butylamino)cyclopentyl)methanol (16-1). After chromatographic purification, a colorless oil was obtained (73%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.38 - 7.29 (m, 15 H), 7.02 - 6.30 (br s, 2H), 4.66 (d, *J* = 11.5 Hz, 1H), 4.60 (d, *J* = 7.9 Hz, 1H), 4.58 (d, *J* = 4.5 Hz, 1H), 4.55 (d, *J* = 8.0 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 8.0 Hz, 1H), 4.27 (t, *J* = 6.8 Hz, 1H), 3.93 (m, 1H), 3.89 (m, 1H), 3.73 (t, *J* = 3.0 Hz, 1H), 3.53 (t, *J* = 7.8 Hz, 1H), 2.93 (m, 2H), 2.68 (m, 1H), 2.02 (s, 2H), 1.62 (m, 2H), 1.28 (m, 2H), 0.87 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 137.70, 137.53, 137.45, 128.52, 128.48, 128.45, 127.96, 127.90, 127.86, 87.58, 84.3, 82.2, 72.4, 72.0, 71.6, 62.4, 61.0, 48.3, 44.0, 29.3, 19.9, 13.6; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{31}H_{40}NO_4$: 490.2957, found 490.2950; IR (neat, NaCl, cm^{-1}): 3308, 3063, 3030, 2927, 2871, 1496, 1454, 1363, 1207, 1089, 1071, 1028, 735, 697; $[\alpha]_D^{25}$ -9.2 (*c* = 0.95, $CHCl_3$).

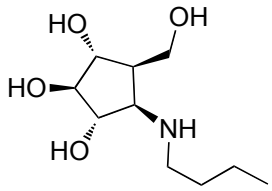


((1*R*,2*R*,3*S*,4*S*,5*R*)-2,3,4-Tris(benzyloxy)-5-(nonylamino)cyclopentyl)methanol (16-2). After chromatographic purification, a colorless oil was obtained (70%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.35 - 7.28 (m, 15 H), 4.70 (d, *J* = 11.8 Hz, 1H), 4.61 (d, *J* = 8.9 Hz, 1H), 4.58 (d, *J* = 6.3 Hz, 1H), 4.56 (d, *J* = 9.3 Hz, 1H), 4.53 (d, *J* = 11.6 Hz, 2H), 3.96 (t, *J* = 4.5 Hz, 1H), 3.82 (m, 4H), 3.26 (t, *J* = 7.3 Hz, 1H), 2.61 (t, *J* = 7.3 Hz, 2H), 2.39 (m, 1H), 1.41 (m, 2H), 1.24 (m, 13H), 0.88 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 138.20, 138.07, 137.94, 128.49, 128.43, 127.84, 127.81, 127.76, 88.4, 85.0, 83.2, 72.06, 72.00, 71.8, 63.3, 62.6, 49.3, 44.4, 31.9, 29.8, 29.51, 29.46, 29.3, 27.1, 22.7, 14.1; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{36}H_{50}NO_4$: 560.3734, found 560.3728; IR (neat, NaCl, cm^{-1}): 3308, 3063, 3030, 2925, 2854, 1496, 1454, 1363, 1089, 1071, 1028, 734, 697; $[\alpha]_D^{25}$ -4.6 (*c* = 0.76, $CHCl_3$).



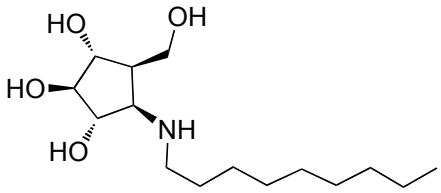
(1*S*,2*S*,3*R*,4*R*,5*R*)-4-(Hydroxymethyl)-5-(alkylamino)cyclopentane-1,2,3-triol (17). Method A: a mixture of the above tri-benzyl derivative, ammonium formate (10 equiv) and 10% palladium on carbon (0.3 g per mmol of *O*-benzyl group) was refluxed in MeOH in a sealed reaction vessel. The reaction was monitored by

thin layer chromatography. Upon consumption of the starting material, the catalyst was carefully filtered off by passing the reaction mixture through a Celite pad. The solvent was subsequently removed under vacuum. The neutral residue was purified by flash column chromatography on silica gel, using 10% MeOH/ CH_2Cl_2 + 1% NH_4OH (v/v) as the eluent. Method B: To a flask, fitted with a dry ice condenser, was charged with anhydrous liquid ammonia, then lithium metal was added until the blue color persisted. A sample of the tri-benzyl derivative in 1,4-dioxane was added dropwise into the flask over 5 min. After an additional 10 min, ammonium chloride was added to the solution until the blue color disappeared. The dry ice condenser was then removed and the ammonia was allowed to evaporate. The residue was extracted with EtOAc (2 x 20 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel with 10% MeOH/ CH_2Cl_2 + 1% NH_4OH (v/v) as the eluent.

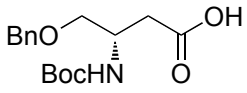


(1*R*,2*S*,3*S*,4*R*,5*R*)-4-(Butylamino)-5-(hydroxymethyl)cyclopentane-1,2,3-triol (17-1). Method A: After chromatographic purification, a colorless viscous oil was obtained (42%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 3.89 (t, *J* = 8.1 Hz, 1H), 3.87 (t, *J* = 3.8 Hz, 1H), 3.77 (dd, *J* = 6.2, 11.4 Hz, 1H), 3.71 (t, *J* = 7.6 Hz, 1H), 3.59 (t, *J* = 7.8 Hz, 1H), 3.50 (t, *J* = 9.2 Hz, 1H), 3.21 (m, 1H), 3.01 (m, 1H), 2.22

(m, 1H), 1.67 (m, 2H), 1.39 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 82.2, 77.8, 75.0, 62.8, 59.6, 48.5, 45.6, 29.3, 20.9, 13.9; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{10}H_{22}NO_4$: 220.1543, found 220.1546; $[\alpha]_D^{25}$ 6.4 (*c* = 0.72, MeOH).



(1*S*,2*S*,3*R*,4*R*,5*R*)-4-(Hydroxymethyl)-5-(nonylamino)cyclopentane-1,2,3-triol (17-2). Method B: After chromatographic purification, a colorless viscous oil was obtained (52%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 3.96 (t, *J* = 8.0 Hz, 1H), 3.93 (dd, *J* = 3.9, 11.8 Hz, 1H), 3.85 (dd, *J* = 6.3, 11.5 Hz, 1H), 3.77 (t, *J* = 7.6 Hz, 1H), 3.65 (t, *J* = 7.7 Hz, 1H), 3.56 (t, *J* = 9.2 Hz, 1H), 3.26 (m, 1H), 3.07 (m, 1H), 2.29 (m, 1H), 1.75 (m, 2H), 1.36 (m, 12H), 0.92 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 80.7, 76.3, 73.5, 61.3, 58.1, 47.2, 44.0, 31.5, 29.0, 28.8, 28.7, 26.1, 25.7, 22.2, 12.9; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{15}H_{32}NO_4$: 290.2331, found 290.2323; $[\alpha]_D^{25}$ 8.8 (*c* = 0.99, MeOH).



(*S*)-4-(Benzyloxy)-3-((*tert*-butoxycarbonyl)amino)butanoic Acid (18). *Warning: Large amounts of diazomethane were used for this transformation. Proper care should be taken when handling this highly explosive reagent. All*

glassware used was free of cracks, scratches or ground-glass joints and a blast shield was used.

N-Boc-*O*-benzyl-D-serine (5.1 g, 17.2 mmol) was dissolved in tetrahydrofuran (50 mL) and cooled to 0 °C in an ice bath. The solution was treated with triethylamine (2.5 mL, 17.2 mmol) and allowed to react for 15 min to fully deprotonate the carboxylic acid. Subsequently, ethyl chloroformate (1.6 mL, 17.2 mmol) was added slowly to the reaction at 0 °C. The resultant anhydride product precipitated out of solution as a thick white precipitate. Stirring was continued for 15 min and then stopped. In a separate flask, an ice-cold solution of diazomethane in ether was prepared (see procedure below) and, without stirring, was carefully transferred into the freshly prepared anhydrous reaction flask using a glass funnel. The reaction solution was lightly stirred for 5 sec, then stirring was stopped. The mixture was allowed to warm to room temperature and react overnight. Any additional diazomethane was carefully quenched with 0.5 N acetic acid.

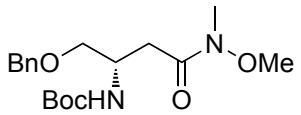
On the following day, the reaction mixture was concentrated *in vacuo*, and the resultant residue was dissolved in chloroform. The solution was washed with 5% sodium bicarbonate (20 mL), 5% HCl (20 mL) and H_2O (20 mL) in sequence. The organic layer was collected, dried over $MgSO_4$, and concentrated *in vacuo*. The resultant crude diazo intermediate was then dissolved in water (7 mL) and tetrahydrofuran (80 mL); cooled to -25 °C and stirred for 30 min. The reaction solution appeared thick and slurry. Aluminum foil was used to cover the reaction flask so as to exclude light from the reaction solution. Silver trifluoroacetate (0.5

g, 10 mol%), dissolved in triethylamine (7.5 mL, 51.6 mmol), was then added. The reaction temperature was allowed to slowly warm to room temperature and the solution was stirred overnight. The reaction mixture was then transferred to a 1 L flask, to which saturated sodium bicarbonate (100 mL) was added and stirred for 1 h. The resultant black solution was partitioned with water and EtOAc. The organic layer with the black suspension was collected and washed with brine once then twice with saturate sodium bicarbonate. The organic phase was set aside and discarded. All aqueous layers were combined in a large Erlenmeyer flask with a large stir bar and cooled to 0 °C. The aqueous solution was then titrated with 5 N HCl until the pH value reached 2. The solution was then extracted with EtOAc (100 mL × 3). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The resultant crude homologated product was purified by flash column chromatography on silica gel, using 30% EtOAc:hexanes (v/v) as the eluent.

Diazomethane preparation: *Warning: Glassware was free of cracks, scratches and ground glass joints.*

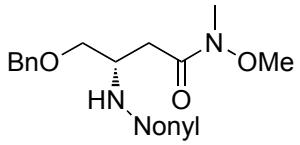
An ice-cold alkaline solution of potassium hydroxide (16.0 g, 2.9 mmol) in H_2O (40 mL) was prepared, and cold ether (200 mL) followed by 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) (7.4 g, 50.0 mmol) was added. The basic solution then turned clear and yellow and bubbled moderately as the reagent dissolved and volatile diazomethane was generated. The solution was reacted for 2 min without stirring. Using a glass funnel, the mixture was transferred into a separatory funnel and the bottom aqueous layer was collected and set in a

negative pressure hood overnight. The yellow organic layer was collected in an ice-cold Erlenmeyer flask, which was pre-charged with potassium hydroxide pellets to absorb any remaining moisture. The diazomethane solution was used immediately after preparation. The remaining aqueous layer was carefully quenched the following day with dilute acid. All other glassware was allowed to sit in a negative pressure hood and was carefully washed with dilute acid. After chromatographic purification, a hygroscopic light green oil was obtained (87%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 10.38 (s, 1H), 7.36 - 7.24 (m, 5 H), 5.32 - 5.30 (br s, 1H), 4.55 (s, 2H), 4.15 (m, 1H), 3.57 (m, 2H), 2.66 (d, *J* = 5.8 Hz, 2H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 176.1, 156.2, 138.4, 128.7, 128.5, 128.04, 128.00, 80.3, 73.6, 71.5, 47.9, 28.7; ESI-HRMS: calc'd *m/e* for [$(M+H^+]$ $C_{16}H_{24}NO_5$: 310.1654, found 310.1651; IR (neat, NaCl, cm^{-1}): 3325, 2978, 2932, 1714, 1500, 1454, 1368, 1250, 1368, 1168, 1117, 1055, 1028, 906, 852, 779, 739, 699; $[\alpha]_D^{25}$ -13 (*c* = 0.93, $CHCl_3$).



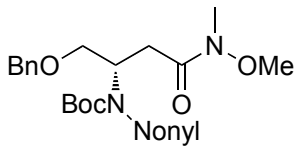
(*S*)-*tert*-Butyl (1-(Benzyloxy)-4-(methoxy(methyl)amino)-4-oxobutan-2-yl)-carbamate (19). Amino acid derivative **18** (812.3 mg, 2.6 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) under a nitrogen atmosphere and cooled to -15 °C. To the solution, *N*,*O*-dimethylhydroxylamine•HCl (389.5 mg, 3.9 mmol) and N-methylmorpholine (0.4 mL, 3.6 mmol) were added in sequence, followed by the addition of EDCI (610.0 mg, 3.1 mmol) in portions over 30 min. The reaction was

then allowed to warm to room temperature. Upon the consumption of the starting material, the reaction was cooled to 0 °C and quenched by the addition of an ice-cold 10% HCl solution (5 mL). The mixture was allowed to stir at 0 °C for 5 min and then diluted with H_2O and extracted with CH_2Cl_2 (20 mL x 3). The organic layers were combined, washed with saturated $NaHCO_3$ (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 2% MeOH/CH_2CH_2 as the eluent. After chromatographic purification, a colorless oil was obtained (91%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.27 (m, 5H), 5.50 (d, *J* = 7.3 Hz, 1H), 4.47 (s, 2H), 4.17 (s, 1H), 3.60 (s, 3H), 3.58 (m, 1H), 3.53 (m, *J* = 6.1 Hz, 1H), 3.10 (s, 3H), 2.82 (d, *J* = 10.5 Hz, 1H), 2.64 (d, *J* = 10.4 Hz, 1H), 1.40 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 173.0, 155.8, 128.8, 128.0, 79.6, 73.5, 71.6, 61.6, 47.5, 33.4, 32.4, 28.8; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{18}H_{29}N_2O_5$: 353.2076, found 353.2066; IR (neat, NaCl, cm^{-1}): 3342, 2976, 2934, 1710, 1654, 1497, 1454, 1390, 1366, 1249, 1170, 1100, 1027, 1001, 856, 739, 699; $[\alpha]_D^{25}$ -7.0 (*c* = 1.1, $CHCl_3$).

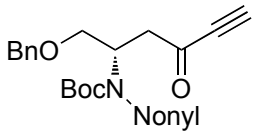


(*S*)-4-(Benzyloxy)-*N*-methoxy-*N*-methyl-3-(nonylamino)butanamide (20). To a flask containing the Weinreb amide derivative **19** (92.3 mg, 0.3 mmol), was added dropwise with 4M HCl in dioxane (2 mL) at 0 °C. The ice bath was removed after the addition was completed. The reaction progress was monitored

by TLC. The solvent was removed *in vacuo* after all starting material was consumed. Dichloroethane (3 mL) was added to the resultant white solid material and neutralized with triethylamine (0.05 mL, 0.3 mmol). To the stirring mixture, nonylaldehyde (45.0 μL, 0.2 mmol) was added and the pH value was adjusted to be slightly acidic with acetic acid. The stirring continued for 30 min, and then sodium triacetoxyborohydride (115.5 mg, 0.5 mmol) was added. The stirring continued at room temperature. Upon consumption of the starting material, the reaction was quenched with saturated sodium bicarbonate (30 mL), and extracted with CH_2Cl_2 (20 mL × 2). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 10% MeOH/CH_2Cl_2 + 1% NH_4OH as the eluent. After chromatographic purification, a light yellow oil was obtained (78%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.31 (m, 5H), 4.52 (s, 2H), 3.66 (s, 3H), 3.55 (m, 2H), 3.30 (t, *J* = 5.6 Hz, 1H), 3.15 (s, 3H), 3.02 - 3.00 (br s, 1H), 2.67 (m, 2H), 2.63 (t, *J* = 7.5 Hz, 2H), 1.49 (t, *J* = 6.6 Hz, 2H), 1.25 (m, 13H), 0.87 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 174.1, 138.6, 128.8, 128.1, 105.0, 73.6, 72.1, 61.7, 54.8, 47.8, 32.3, 30.4, 30.1, 30.0, 29.9, 29.7, 27.7, 23.1, 14.5; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{22}H_{39}N_2O_3$: 379.2961, found 379.2958; IR (neat, NaCl, cm^{-1}): 2925, 2854, 1720, 1664, 1495, 1454, 1379, 1273, 1366, 1170, 1101, 1026, 1001, 735, 698; $[\alpha]_D^{25}$ -10 (*c* = 0.83, $CHCl_3$).

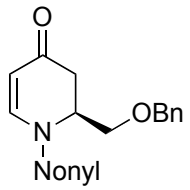


(*S*)-*tert*-Butyl (1-(Benzyloxy)-4-(methoxy(methyl)amino)-4-oxobutan-2-yl)(nonyl)carbamate (21). The secondary amine **20** (77.8 mg, 0.2 mmol) was dissolved in anhydrous acetonitrile (10 mL). To the solution, di-*tert*-butyl dicarbonate (297.3 mg, 1.2 mmol) and dimethylaminopyridine (61.4 mg, 0.5 mmol) were added in sequence. The mixture was stirred at room temperature overnight. The reaction was then quenched by addition of saturated ammonium chloride (20 mL), and extracted with EtOAc (20 mL × 3). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 20% EtOAc/hexanes. After chromatographic purification, a colorless oil was obtained (68%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.30 (m, 5H), 4.50 (s, 2H), 4.15 (m, 1H), 3.70 (m, 2H), 3.64 (s, 3H), 3.21 (m, 2H), 3.14 (s, 3H), 3.06 (m, 1H), 2.75 (m, 1H), 1.51 (s, 2H), 1.42 (s, 9H), 1.24 (m, 12H), 0.87 (t, *J* = 5.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 172.0, 150.8, 128.2, 127.4, 82.6, 72.8, 31.9, 29.88, 29.86, 29.7, 29.54, 29.48, 29.3, 28.4, 28.0, 27.7, 23.9, 22.7, 14.1; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{27}H_{47}N_2O_5$: 501.3304, found 501.3311; IR (neat, NaCl, cm^{-1}): 2926, 2855, 1778, 1742, 1690, 1649, 1458, 1421, 1367, 1230, 1148; $[\alpha]_D^{25}$ -3.6 (*c* = 0.37, $CHCl_3$).



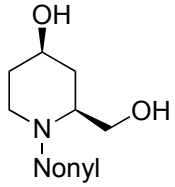
(*S*)-*tert*-Butyl (1-(Benzyloxy)-4-oxohex-5-yn-2-yl)(nonyl)carbamate (22). Weinreb amide **21** (341.2 mg, 0.7 mmol) was dissolved in anhydrous THF (15

mL) under a nitrogen atmosphere and cooled to 0 °C. To the stirring solution, a 0.5M solution of ethynylmagnesium bromide (7.5 mL, 3.5 mmol) in THF was added dropwise. The reaction was then allowed to warm to room temperature. The reaction was monitored by TLC. Upon consumption of the starting material, the reaction was quenched with an ice-cold 10% HCl solution (5 mL) and allowed to stir at 0 °C for 5 min. The mixture was then diluted with water and extracted with EtOAc (30 mL x 3). The organic layers were combined, washed with saturated sodium bicarbonate solution (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 10% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (82%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.31 (m, 5H), 4.50 (s, 2H), 4.33 (m, 1H), 3.62 (m, 2H), 3.14 (m, 5H), 1.44 (m, 11H), 1.26 (m, 12H), 0.90 (m, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 128.3, 127.5, 81.6, 78.7, 75.7, 72.9, 53.2, 39.7, 31.9, 29.85, 29.81, 29.7, 29.53, 29.48, 29.3, 28.4, 27.7, 22.7, 14.1; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{27}H_{42}NO_4$: 466.2933, found 466.2926; IR (neat, NaCl, cm^{-1}): 2926, 2855, 2092, 1690, 1455, 1366, 1247, 1168, 1100; $[\alpha]_D^{25}$ -9.0 (*c* = 0.70, $CHCl_3$).



(*S*)-2-((Benzyloxy)methyl)-1-nonyl-2,3-dihydropyridin-4(1*H*)-one (23). Ynone **22** (143.1 mg, 0.8 mmol) was dissolved in a 4 N HCl-dioxane solution (6 mL, 2.4

mmol) and allowed to react for 15 min. The dioxane and excess HCl were removed under reduced pressure and the residue was placed under high vacuum for another 15 min. This material was then dissolved in MeOH (10 mL) and excess potassium carbonate (8.0 mmol) was added. The reaction was monitored by TLC. Upon consumption of the starting material, the reaction mixture was diluted with CH_2Cl_2, and the inorganic salts were filtered off. The organic phase was concentrated *in vacuo*, the resultant residue was purified by flash column chromatography on silica gel, using 60% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a yellow oil was obtained (92%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.36 - 7.28 (m, 5H), 6.95 (d, *J* = 7.4 Hz, 1H), 4.88 (d, *J* = 7.3 Hz, 1H), 4.51 (s, 2H), 3.80 (dd, *J* = 8.8, 16.2 Hz, 1H), 3.77 (m, 1H), 3.51 (dd, *J* = 4.0, 8.9 Hz, 1H), 3.42 (m, 1H), 3.20 (m, 1H), 2.77 (dd, *J* = 9.7, 16.7 Hz, 1H), 2.42 (dd, *J* = 2.2, 16.5 Hz, 1H), 1.60 (t, *J* = 5.8 Hz, 2H), 1.28 (m, 12H), 0.90 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 189.9, 152.5, 137.7, 128.4, 127.6, 96.4, 73.5, 68.3, 56.0, 55.2, 37.7, 31.8, 29.7, 29.4, 29.21, 29.18, 26.5, 22.6, 14.1; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{22}H_{34}NO_2$: 366.2409, found 366.2415; IR (neat, NaCl, cm^{-1}): 3031, 2926, 2855, 1641, 1589, 1497, 1454, 1411, 1375, 1350, 1288, 1220, 1177, 1115, 1028, 1025, 739, 698; $[\alpha]_D^{25}$ -81 (*c* = 1.0, $CHCl_3$).

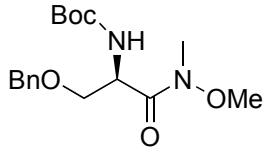


(2*S*,4*R*)-2-(Hydroxymethyl)-1-nonylpiperidin-4-ol (24). Method A: Enaminone **23** (72.2 mg, 0.2 mmol) was dissolved in anhydrous ethanol (3 mL). To the stirring solution, sodium borohydride (120.0 mg, 2.2 mmol) was added. The mixture was allowed to stir for 48 h. Upon the disappearance of the starting material on TLC, the solvent was removed *in vacuo*. The resultant residue was dissolved in CH_2Cl_2 and washed with H_2O; the aqueous layer was extracted with CH_2Cl_2 (20 mL × 2). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude material (59.2 mg) was dissolved in anhydrous MeOH (3 mL). To the stirring solution, 10 wt% Pd/C (60.0 mg) was added carefully. The mixture was allowed to react under hydrogen gas at 1 atmosphere overnight. Upon consumption of the starting material, the catalyst was filtered off by passing the reaction through a Celite pad, and then the filtrate was concentrated *in vacuo*. The resultant residue was purified by flash column chromatography on silica gel, using 10% MeOH/CH_2Cl_2 + 1% NH_4OH as the eluent. After chromatographic purification, a viscous light yellow oil was obtained (45%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.89 (dd, *J* = 3.8, 11.1 Hz, 1H), 3.70 (m, 1H), 3.43 (dd, *J* = 1.9, 10.9 Hz, 1H), 3.12 (dt, *J* = 3.4, 12 Hz, 1H), 2.77 (dt, *J* = 5.0, 12.4 Hz, 1H), 2.43 (s, 1H), 2.30 (m, 2H), 1.94 (m, 2H), 1.72 (d, *J* = 10.8 Hz, 1H), 1.72 - 1.60 (br s, 2H), 1.66 (d, *J* = 10.7 Hz, 1H), 1.50 (m, 2H), 1.28 (m, 12H), 0.90 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 68.8, 63.1, 60.1, 52.9, 50.1, 37.9, 34.6, 32.3, 30.01, 29.98, 29.7, 28.0, 26.3, 23.1, 14.5; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{15}H_{32}NO_2$: 258.2433, found 258.2427; IR (neat, NaCl, cm^{-1}): 3031, 2926, 2855, 1641, 1589, 1497, 1454, 1411, 1375, 1350, 1288,

1220, 1177, 1115, 1028, 1025, 739, 698; $[\alpha]_D^{25}$ -22 (*c* = 0.75, MeOH).

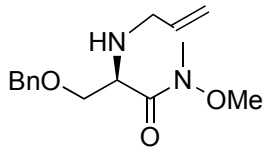
Method B: (2*S*,4*R*)-1-*tert*-Butyl 2-methyl 4-hydroxypiperidine-1,2-dicarboxylate (181.8 mg, 0.67 mM) was added to a dry flask and cooled to 0 °C. To the flask was added 4 N HCl in dioxane (5 mL, 20 mM). The reaction was then warmed to room temperature and the stirring continued for 30 min. Solvent was then removed *in vacuo* and the crude material was used in the reductive alkylation without purification. The HCl salt was dissolved in dichloroethane (5 mL) at room temperature. To the flask, triethylamine (0.2 mL, 1.43 mM), sodium triacetoxyborohydride (203.0 mg, 0.96 mM) and nonylaldehyde (0.7 mL, 0.85 mM) were added in sequence. pH value of the reaction was adjusted to < 7 with acetic acid. The stirring continued for 2 h, upon consumption of the starting material, the reaction was quenched with saturated sodium bicarbonate (20 mL), and extracted with CH_2Cl_2 (10 mL × 2). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 10% MeOH/CH_2Cl_2 + 1% NH_4OH as the eluent. After chromatographic purification, a light green oil was obtained (80%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.85 (s, 3H), 3.12 (m, 1H), 2.50 (m, 1H), 2.25 (m, 1H), 2.16 (m, 1H), 2.09 (d, *J* = 12.1 Hz, 1H), 1.92 (d, *J* = 10.7 Hz, 1H), 1.78 (m, 1H), 1.67 (m, 1H), 1.49 (s, 2H), 1.26 (s, 14H), 0.88 (t, *J* = 6.4 Hz, 3H). The *N*-alkylated methyl ester (130.0 mg, 0.46 mM) was dissolved in toluene (5 mL) and cooled to -60 °C. To the stirring solution, DIBAL (1M in THF, 4.5 mL, 4.5 mM) was added dropwise. The stirring continued for 4 h and the reaction was warmed to 0 °C. Then ether (30 mL) was added and the stirring

continued for 15 min at 0 °C. H_2O (0.15 mL), 25% NaOH solution (75 μL) and H_2O (0.30 mL) were added to the flask in sequence. The aluminum salts were filtered, and the solvent was removed *in vacuo* for the filtrate. The crude material was purified by flash column chromatography on silica gel, using 10% MeOH/CH_2Cl_2 + 1% NH_4OH as the eluent. After chromatographic purification, a viscous light yellow oil was obtained (40%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.89 (dd, *J* = 3.8, 11.1 Hz, 1H), 3.70 (m, 1H), 3.43 (dd, *J* = 1.9, 10.9 Hz, 1H), 3.12 (dt, *J* = 3.4, 12 Hz, 1H), 2.77 (dt, *J* = 5.0, 12.4 Hz, 1H), 2.43 (s, 1H), 2.30 (m, 2H), 1.94 (m, 2H), 1.72 (d, *J* = 10.8 Hz, 1H), 1.72 - 1.60 (br s, 2H), 1.66 (d, *J* = 10.7 Hz, 1H), 1.50 (m, 2H), 1.28 (m, 12H), 0.90 (t, *J* = 6.5 Hz, 3H).



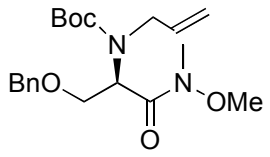
(*R*)-*tert*-Butyl (3-(Benzyloxy)-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate (25). *N*-Boc-*O*-benzyl-D-serine (500.0 mg, 1.7 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) under a nitrogen atmosphere and cooled to -15 °C. To the stirring solution, *N*,*O*-dimethylhydroxylamine•HCl (214.0 mg, 2.2 mmol) and *N*-methylmorpholine (0.3 mL, 2.4 mmol) were added, followed by addition of EDCI (454.4 mg, 2.4 mmol) in portions over 30 min. The reaction was then allowed to warm to room temperature. Upon consumption of the starting material, the reaction was cooled to 0 °C and quenched by the addition of an ice-cold 10% HCl solution (5 mL). The mixture was allowed to stir at 0 °C for 5 min and was then diluted with H_2O and extracted with CH_2Cl_2 (20 mL x 3). The

organic layers were combined, washed with saturated $NaHCO_3$ (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 25% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (90%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.31 (m, 5H), 5.42 (d, *J* = 9.2 Hz, 1H), 4.88 (s, 1H), 4.57 (d, *J* = 12.3 Hz, 1H), 4.50 (d, *J* = 12.2 Hz, 1H), 3.71 (s, 3H), 3.68 (t, *J* = 4.8 Hz, 2H), 3.21 (s, 3H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 155.7, 128.4, 127.69, 127.65, 73.1, 70.0, 64.0, 61.5, 50.8, 37.9, 28.4; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{17}H_{27}N_2O_5$: 339.1920, found 339.1926; IR (neat, NaCl, cm^{-1}): 3326, 2977, 2936, 2868, 1713, 1667, 1497, 1455, 1391, 1366, 1250, 1169, 1108, 1052, 1024, 989, 865, 740, 699; $[\alpha]_D^{25}$ -3.9 (*c* = 0.97, $CHCl_3$).



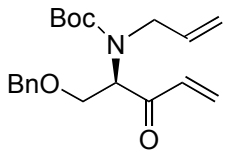
(*R*)-2-(Allylamino)-3-(benzyloxy)-*N*-methoxy-*N*-methylpropanamide (26). To a flask containing the Weinreb amide **25** (525.0 mg, 1.6 mmol), 4M HCl in dioxane (5 mL, 20.0 mmol) was added dropwise at 0 °C. The ice bath was removed after the addition was completed. The reaction was monitored by TLC. The solvent was removed *in vacuo* after the starting material was consumed. The resultant amorphous solid material was then suspended in anhydrous MeOH (8 mL), basified with diisopropylethylamine (0.8 mL, 4.8 mmol) dropwise. The mixture turned into a clear solution. To the solution, allylbromide (0.3 mL, 3.2

mmol) was added slowly, and allowed to react at 50 °C for 6 h. Upon consumption of the starting material, the reaction was quenched with saturated ammonium chloride (15 mL), and extracted with CH_2Cl_2 (20 mL × 2). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resultant crude material was purified by flash column chromatography on silica gel, using 5% MeOH/CH_2Cl_2 + 1% NH_4OH as the eluent. After chromatographic purification, a colorless oil was obtained (50%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.34 - 7.23 (m, 5H), 5.87 (m, 1H), 5.18 (ddd, *J* = 1.6, 3.2, 17.2 Hz, 1H), 5.08 (dd, *J* = 1.5, 10.2 Hz, 1H), 4.53 (s, 2H), 3.96 (s, 1H), 3.66 (s, 3H), 3.60 (m, 2H), 3.28 (ddt, *J* = 1.5, 5.8, 13.8 Hz, 1H), 3.22 (s, 3H), 3.12 (ddt, *J* = 1.2, 6.3, 13.8 Hz, 1H), 2.04 (m, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 172.8, 138.1, 136.7, 128.3, 127.62, 127.57, 116.3, 73.4, 71.3, 61.5, 56.7, 50.7, 34.8; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{15}H_{23}N_2O_3$: 279.1709, found 279.1715; IR (neat, NaCl, cm^{-1}): 3323, 2975, 2936, 2866, 1711, 1662, 1496, 1454, 1389, 1366, 1251, 1173, 1107, 1028, 992, 922, 864, 821, 740, 700; $[\alpha]_D^{25}$ 6.0 (*c* = 0.50, $CHCl_3$).



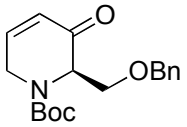
(*R*)-*tert*-Butyl Allyl(3-(benzyloxy)-1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate (27). The secondary amine **26** (180.0 mg, 0.6 mmol) was dissolved in freshly distilled EtOAc (10 mL). To the stirring solution, di-*tert*-butyl dicarbonate (0.2 mL, 0.8 mmol) was added slowly. The reaction was stirred at

room temperature for 36 h and monitored by TLC. Upon consumption of the starting material, the reaction was quenched by addition of saturated ammonium chloride (20 mL), and extracted with EtOAc (20 mL × 3). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 50% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (94%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.32 - 7.26 (m, 5H), 5.80 (m, 1H), 5.48 (s, 1H), 5.10 (m, 1H), 5.03 (t, *J* = 10.1 Hz, 1H), 4.59 (d, *J* = 12.0 Hz, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 3.92 (m, 1H), 3.85 (d, *J* = 5.7 Hz, 1H), 3.81 (d, *J* = 5.6 Hz, 1H), 3.72 (s, 3H), 3.60 (s, 1H), 3.17 (s, 3H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 197.9, 155.5, 138.1, 135.7, 135.0, 128.3, 127.6, 115.7, 80.1, 72.8, 67.5, 62.3, 53.4, 46.7, 31.5, 28.3; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{20}H_{31}N_2O_5$: 379.2233, found 379.2229; IR (neat, NaCl, cm^{-1}): 2976, 2934, 2868, 1695, 1671, 1454, 1400, 1366, 1318, 1270, 1252, 1174, 1150, 1104, 1029, 994, 922, 863, 829, 738, 699; $[\alpha]_D^{25}$ 69 (*c* = 0.52, $CHCl_3$).



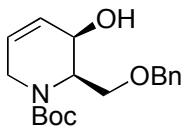
(*R*)-*tert*-Butyl Allyl(1-(benzyloxy)-3-oxopent-4-en-2-yl)carbamate (28). Weinreb amide **27** (230.0 mg, 0.6 mmol) was dissolved in anhydrous THF (15 mL) under a nitrogen atmosphere and cooled to 0 °C. To the stirring solution, a 1.0M solution of vinylmagnesium bromide (3.1 mL, 3.1 mmol) in THF was added dropwise. The reaction was then allowed to warm to room temperature. The

reaction was monitored by TLC. Upon consumption of the starting material, the reaction was quenched with an ice-cold 10% HCl solution (5 mL) and allowed to stir at 0 °C for 5 min. The reaction was diluted with water and extracted with EtOAc (30 mL x 3). The organic layers were combined, washed with saturated sodium bicarbonate solution (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 20% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (90%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.34 - 7.27 (m, 5H), 6.59 (m, 1H), 6.37 (dd, *J* = 5.9, 16.6 Hz, 1H), 5.92 (m, 1H), 5.72 (t, *J* = 10.4 Hz, 1H), 5.20 (t, *J* = 9.8 Hz, 1H), 5.08 (t, *J* = 10.2 Hz, 1H), 4.76 (d, *J* = 6.0 Hz, 0.4H), 4.58 (d, *J* = 10.9 Hz, 1H), 4.49 (d, *J* = 11.5 Hz, 1H), 4.33 (dd, *J* = 4.6, 15.0 Hz, 0.6H), 4.10 (t, *J* = 7.2 Hz, 1H), 3.96 (m, 1H), 3.79 (m, 2H), 1.45 (s, 4H), 1.25 (s, 5H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 196.5, 195.6, 155.4, 154.3, 138.0, 134.8, 134.0, 133.2, 132.2, 128.9, 128.5, 128.4, 128.3, 127.6, 127.5, 118.6, 116.7, 81.5, 80.6, 73.3, 73.1, 68.7, 67.6, 64.2, 62.1, 51.1, 49.1, 28.3, 28.1; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{20}H_{28}NO_4$: 368.1853, found 368.1862; IR (neat, NaCl, cm^{-1}): 2977, 2930, 2868, 1699, 1614, 1454, 1401, 1367, 1251, 1169, 1152, 1103, 1028, 987, 773, 737, 698; $[\alpha]_D^{25}$ 180 (*c* = 0.90, $CHCl_3$).



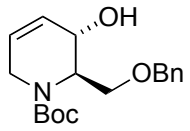
(*R*)-*tert*-Butyl 6-((Benzyloxy)methyl)-5-oxo-5,6-dihydropyridine-1(2*H*)-

carboxylate (29). To a solution of diene **28** (131.1 mg, 0.4 mmol) in CH_2Cl_2 (5 mL), the second generation of Grubbs catalyst was added (32 mg, 10 mol%). After 2 h under reflux, the solvent was removed *in vacuo*, and the crude residue was purified by flash column chromatography on silica gel, using 25% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (75%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.31 (m, 3H), 7.23 (m, 2H), 7.00 (m, 1H), 6.19 (d, *J* = 10.4 Hz, 1H), 4.68 (m, 2H), 4.46 (s, 2H), 4.03 (m, 1H), 3.82 (d, *J* = 8.6 Hz, 1H), 3.68 (d, *J* = 7.4 Hz, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 194.7, 154.3, 147.5, 146.4, 137.8, 128.4, 127.6, 127.2, 127.0, 80.9, 73.1, 71.4, 61.1, 59.8, 43.3, 42.3, 28.3; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{18}H_{23}NNaO_4$: 340.1525, found 340.1543; IR (neat, NaCl, cm^{-1}): 2976, 2930, 2863, 1685, 1475, 1454, 1412, 1381, 1367, 1353, 1311, 1238, 1169, 1111, 1021, 938, 860, 737, 697; $[\alpha]_D^{25}$ -68 (*c* = 1.1, $CHCl_3$).



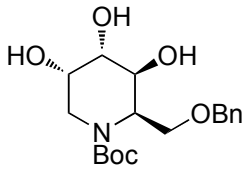
(5*R*,6*R*)-*tert*-Butyl 6-((Benzyloxy)methyl)-5-hydroxy-5,6-dihydropyridine-1(2*H*)-carboxylate (30). Enone **29** (134.0 mg, 0.4 mmol) was dissolved in anhydrous MeOH (8 mL), and cooled to -78 °C with a dry ice/acetone bath. To the stirring solution, cerium chloride (156.0 mg, 0.6 mmol) was added. After 15 min, sodium borohydride (18.0 mg, 0.5 mmol) was added to the solution. The reaction was completed within 15 min after the addition. The excess sodium borohydride was quenched with an ice-cold 10% HCl solution. The aqueous

layer was extracted with EtOAc (20 mL × 3). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resultant residue was purified by flash column chromatography on silica gel, using 50% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (92%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.33 - 7.29 (m, 5H), 5.75 (d, *J* = 10.4 Hz, 1H), 5.66 (m, 1H), 5.00 - 4.60 (br s, 1H), 4.56 (d, *J* = 11.9 Hz, 1H), 4.53 (m, 1H), 4.48 (d, *J* = 11.9 Hz, 1H), 4.09 (m, 1H), 3.78 (dd, *J* = 2.3, 9.7 Hz, 1H), 3.54 (t, *J* = 7.2 Hz, 1H), 3.40 (d, *J* = 19.3 Hz, 1H), 3.13 (m, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 154.8, 137.7, 128.7, 128.4, 127.8, 127.6, 123.7, 80.2, 73.2, 67.2, 66.7, 40.5, 28.4; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{18}H_{25}NNaO_4$: 342.1681, found 340.1700; IR (neat, NaCl, cm^{-1}): 3430, 2929, 2977, 2863, 1694, 1676, 1477, 1454, 1415, 1366, 1316, 1249, 1170, 1121, 1076, 1003, 941, 911, 856, 736, 698; $[\alpha]_D^{25}$ - 6.0 (*c* = 0.55, $CHCl_3$).



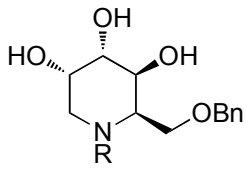
(5*S*,6*R*)-*tert*-Butyl 6-(Benzyloxymethyl)-5-hydroxy-5,6-dihydropyridine-1(2*H*)-carboxylate (5-*epi*-30). Allylic alcohol **30** (54.3 mg, 0.17 mM) was dissolved in THF (6 mL). To the stirring solution, triphenylphosphine (81 mg, 0.31 mM), *p*-NO_2-benzoic acid (35.0 mg, 0.20 mM) and diisopropyl azodicarboxylate (65 μL, 0.31 mM) were added in sequence. The stirring continued for 2 h at room temperature. The reaction was then quenched with with H_2O (20 mL), the aqueous layer was extracted with EtOAc (20 mL × 3). The

organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 20% EtOAc:hexanes (v/v) as the eluent. The resultant intermediate (64.3 mg, 0.14 mM, 81%) was then dissolved in MeOH (5 mL). To the stirring solution, K_2CO_3 (100 mg, 0.69 mM) was added. The stirring continued until all the starting material was consumed. The salt was then filtered, and the filtrate was concentrated *in vacu*o. The crude material was purified by flash column chromatography on silica gel, using 50% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (60%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.31 (m, 5H), 5.94 (m, 1H), 5.88 (s, 1H), 4.56 (d, *J* = 12.0 Hz, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.35 - 4.18 (br m, 1H), 4.11 (s, 1H), 3.48 (d, *J* = 19.6 Hz, 1H), 3.40 (d, *J* = 6.2 Hz, 2H), 1.70 - 1.60 (br s 1H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 153.2, 138.9, 128.5, 128.3, 127.6, 127.5, 121.8, 86.2, 72.6, 68.0, 63.0, 43.0, 28.4; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{18}H_{25}NNaO_4$: 342.1681, found 340.1700.



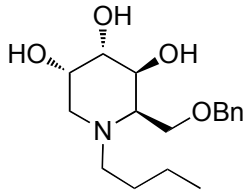
(2*R*,3*S*,4*S*,5*S*)-*tert*-Butyl 2-((Benzyloxy)methyl)-3,4,5-trihydroxypiperidine-1-carboxylate (31). Allyl alcohol **30** (103.0 mg, 0.3 mmol) was dissolved in CH_2Cl_2 (3 mL). To the stirring solution, a solution of *N*-methylmorpholine *N*-oxide (116.0 mg, 1.0 mmol) in H_2O (0.23 mL) was added and the mixture was cooled to 0 °C.

To the mixture, OsO_4 (0.17 mL, 2.5 wt%, 5 mol%) was added and the reaction was allowed to warm to room temperature and was stirred for 12 h. Upon consumption of the starting material, the reaction was diluted with saturated ammonium chloride (10 mL) and extracted with CH_2Cl_2 (20 mL × 2). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 10% MeOH/CH_2Cl_2 as the eluent. After chromatographic purification, a colorless oil was obtained (78%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 - 7.29 (m, 5H), 4.80 - 4.60 (br s, 1H), 4.54 (d, *J* = 12.2 Hz, 1H), 4.50 (d, *J* = 12.0 Hz, 1H), 4.30 - 4.10 (br s, 1H), 3.99 (dd, *J* = 3.5, 9.9 Hz, 2H), 3.81 (m, 1H), 3.77 (d, *J* = 5.3 Hz, 2H), 3.15 (d, *J* = 14.9 Hz, 1H), 2.82 (m, 1H), 2.55 (m, 1H), 2.25 - 2.10 (br s, 1H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 156.0, 138.0, 128.4, 127.6, 127.5, 80.5, 73.6, 73.2, 71.9, 68.5, 68.3, 66.5, 44.6, 28.4; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{18}H_{27}NNaO_6$: 376.1736, found 376.1735; IR (neat, NaCl, cm^{-1}): 3407, 2976, 2928, 1668, 1477, 1454, 1427, 1366, 1248, 1171, 1139, 1081, 948, 858, 818, 790, 738, 698; $[\alpha]_D^{25}$ - 0.90 (*c* = 1.0, $CHCl_3$).



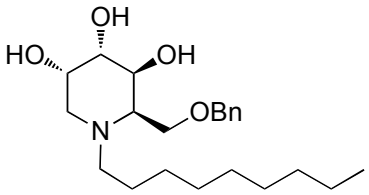
General Procedure for the Synthesis of (2*R*,3*S*,4*S*,5*S*)-1-Alkyl-2-((benzyloxy)methyl)-piperidine-3,4,5-triols (32). The triol derivative **31** was dissolved in formic acid (10 mL per 1 mmol of starting material). The mixture

was allowed to stir for 3 h at room temperature. Upon consumption of the starting material, the solvent was removed *in vacuo*, and the remaining residue was left on high vacuum for 1 h. The residue was then charged with dichloroethane (20 mL per 1 mmol of starting material). To the stirring suspension, was added triethylamine (2.0 equiv relative to the starting material) and the corresponding aldehyde (1.5 equiv). The mixture was allowed to stir for 15 min until it turned into a clear solution. To the stirring solution, sodium triacetoxyborohydride (2.0 equiv) was added. Acetic acid was added to adjust the pH of the reaction to be slightly acidic. The mixture was allowed to react at room temperature overnight. The reaction was then quenched with saturated sodium bicarbonate (10 mL) and extracted with CH_2Cl_2 (2 x 20 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 10% MeOH/CH_2Cl_2 + 1% NH_4OH as the eluent.

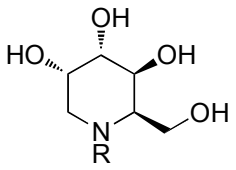


(2*R*,3*S*,4*S*,5*S*)-2-((Benzyloxy)methyl)-1-butylpiperidine-3,4,5-triol (32-1). After chromatographic purification, a colorless oil was obtained (73%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 7.37 - 7.30 (m, 5H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.03 (m, 1H), 3.94 (dd, *J* = 2.5, 5.0 Hz, 1H), 3.80 (dd, *J* = 1.6, 4.6 Hz, 1H), 3.77 (d, *J* = 4.3 Hz, 2H), 3.17 (m, 1H), 2.81 (m, 4H), 1.54 (m, 2H), 1.25 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 139.2,

129.4, 129.1, 128.9, 74.3, 72.4, 71.6, 69.8, 66.3, 60.1, 54.8, 52.6, 27.1, 21.5, 14.2; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{17}H_{28}NO_4$: 310.2018, found 310.2003; IR (neat, NaCl, cm^{-1}): 3380, 2956, 2929, 2863, 1454, 1366, 1092, 1073, 734, 697; $[\alpha]_D^{25}$ 7.6 (*c* = 0.84, $CHCl_3$).

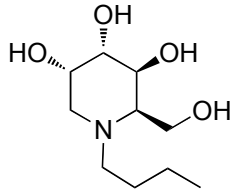


(2*R*,3*S*,4*S*,5*S*)-2-((Benzyloxy)methyl)-1-nonylpiperidine-3,4,5-triol (32-2). After chromatographic purification, a colorless oil was obtained (71%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 7.36 - 7.30 (m, 5H), 4.56 (d, *J* = 11.8 Hz, 1H), 4.51 (d, *J* = 11.8 Hz, 1H), 3.99 (t, *J* = 4.8 Hz, 1H), 3.91 (dd, *J* = 2.4, 4.8 Hz, 1H), 3.78 (t, *J* = 3.3 Hz, 1H), 3.75 (d, *J* = 4.7 Hz, 2H), 3.06 (m, 1H), 2.75 (m, 4H), 1.54 (m, 2H), 1.28 (m, 12H), 0.92 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 129.4, 129.0, 128.8, 74.3, 72.4, 71.6, 69.8, 66.3, 60.1, 54.8, 52.6, 35.8, 33.1, 30.7, 30.6, 30.4, 28.5, 23.7, 14.4; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{22}H_{37}NNaO_4$: 402.2620, found 402.2607; IR (neat, NaCl, cm^{-1}): 3339, 2925, 2854, 1454, 1366, 1257, 1093, 1070, 733, 694; $[\alpha]_D^{25}$ 6.9 (*c* = 0.36, $CHCl_3$).

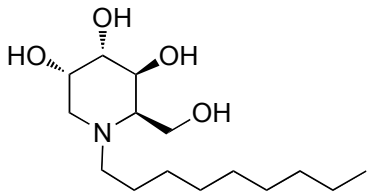


(2*R*,3*S*,4*S*,5*S*)-1-Alkyl-2-(hydroxymethyl)-piperidine-3,4,5-triol (33). A

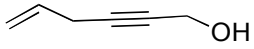
mixture of the tertiary amine derivatives (**32**), ammonium formate (10 equiv) and 10% palladium on carbon (0.3 g per mmol of *O*-benzyl group) was refluxed in MeOH in a sealed reaction vessel. The reaction was monitored by thin layer chromatography. After the starting material was consumed, the catalyst was carefully filtered off by passing the reaction mixture through a Celite pad. The solvent was subsequently removed under vacuum. The neutral residue was purified by flash column chromatography, using 10% MeOH/ CH_2Cl_2 + 1% NH_4OH (v/v) as the eluent.



(2*R*,3*S*,4*S*,5*S*)-1-Butyl-2-(hydroxymethyl)piperidine-3,4,5-triol (33-1). After chromatographic purification, a colorless viscous oil was obtained (66%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 4.22 (dt, *J* = 2.8, 8.0 Hz, 1H), 4.08 (m, 1H), 3.97 (d, *J* = 3.8 Hz, 2H), 3.90 (dd, *J* = 1.7, 4.5 Hz, 1H), 3.50 (m, 1H), 3.29 (d, *J* = 8.4 Hz, 2H), 3.21 (d, *J* = 9.8 Hz, 2H), 1.76 (m, 2H), 1.44 (m, 2H), 1.03 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 72.0, 70.2, 64.1, 61.9, 60.5, 55.0, 51.1, 27.7, 21.0, 13.9; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{10}H_{22}NO_4$: 220.1549, found 220.1567; $[\alpha]_D^{25}$ -7.0 (*c* = 0.59, MeOH).

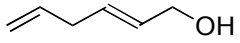


(2*R*,3*S*,4*S*,5*S*)-2-(Hydroxymethyl)-1-nonylpiperidine-3,4,5-triol (33-2). After chromatographic purification, a colorless viscous oil was obtained (88%). 1H NMR (MeOD, 400 MHz, ppm): δ 4.24 (dt, *J* = 2.6, 7.8 Hz, 1H), 4.10 (d, *J* = 3.0 Hz, 1H), 3.98 (d, *J* = 4.2 Hz, 2H), 3.92 (dd, *J* = 1.4, 6.2 Hz, 1H), 3.50 (m, 1H), 3.30 (d, *J* = 8.6 Hz, 2H), 3.22 (d, *J* = 8.0 Hz, 2H), 1.79 (m, 2H), 1.40 - 1.34 (m, 12H), 0.93 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 71.9, 70.2, 64.0, 61.9, 60.7, 54.8, 51.1, 33.0, 30.5, 30.31, 30.25, 27.7, 23.7, 14.4; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{15}H_{32}NO_4$: 290.2331, found 290.2321; $[\alpha]_D^{25}$ -13 (*c* = 0.84, MeOH).

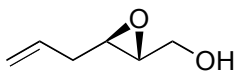


Hex-5-en-2-yn-1-ol. To a brine solution (120 mL), HCl (0.5 mL), copper bromide (5.8 g, 40.4 mmol) and propargyl alcohol (14.0 g, 249.7 mmol) were added in sequence at room temperature. The pH value of the mixture was adjusted to 9 with a NaOH solution (40% v/v, 15 mL). The mixture was then heated to 70 °C, and a solution of allyl bromide in MeOH (64 : 40, v/v) was added dropwise via a syringe pump. The pH value was kept between 8 and 9 with NaOH throughout the addition. The mixture was stirred at 70 °C for 3.5 h after the addition was finished, and then cooled to room temperature. The pH value was re-adjusted to 2 with HCl. The mixture was extracted with ether (50 mL × 3), and the organic

layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by a bulb-to-bulb distillation (bp 68 - 70 °C, 10 mmHg), to give a colorless water-like liquid (84%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.81 (m, 1H), 5.32 (dd, *J* = 1.7, 17 Hz, 1H), 5.13 (dd, *J* = 1.6, 10 Hz, 1H), 4.30 (t, *J* = 2.2 Hz, 2H), 3.01 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 132.2, 116.3, 83.0, 80.6, 51.4, 23.1.

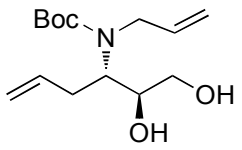


(*E*)-Hexa-2,5-dien-1-ol. To a stirring mixture of lithium aluminum hydride (3.8 g, 100.1 mmol) in THF (150 mL), a solution of hex-5-en-2-yn-1-ol (9.6 g, 99.9 mmol) in THF (50 mL) was added dropwise at 0 °C. Upon completion of the addition, the reaction was allowed to warm to room temperature and stirred for 30 min. The reaction was heated to 45 °C for another 3 h and then cooled to 0 °C again. The mixture was quenched with a saturated ammonium chloride solution (100 mL) and extracted with ether (20 mL × 3). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by distillation (bp 70 - 72 °C, 10 mmHg), to give a colorless oil (37%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.82 (m, 2H), 5.71 (m, 1H), 5.04 (m, 2H), 4.12 (dd, *J* = 1.4, 2.5 Hz, 2H), 2.81 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 136.3, 130.6, 130.0, 115.6, 63.7, 36.3.



((2*R*,3*R*)-3-Allyloxiran-2-yl)methanol. (*E*)-Hexa-2,5-dien-1-ol (3.3 g, 33.4 mmol)

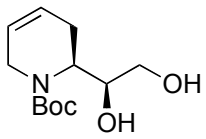
was dissolved in anhydrous CH_2Cl_2 (120 mL) and cooled to -25 °C. To the stirring solution, powdered 4 Å molecular sieves (1.0 g), diethyl-D-(-)-tartrate (0.7 mL, 3.3 mmol) and titanium tetraisoproxide (1.0 mL, 3.3 mmol) were added. The mixture was stirred at -25 °C for 30 min. After the "aging", a solution of cumene hydroperoxide (11 mL, 66.8 mmol) in CH_2Cl_2 (10 mL) was added to the reaction. The mixture was stirred at -20 °C for 12 h. The reaction was quenched with a 10% solution (3 mL) of NaOH saturated by NaCl (prepared by dissolving 10 g NaCl and 10 g NaOH in 95 mL of H_2O, and pre-cooled). Then the cooling bath was removed, and the reaction was warmed to 10 °C for 10 min. The mixture was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, using 60% ether:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (60%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.82 (m, 1H), 5.18 (dd, *J* = 1.6, 3.2 Hz, 1H), 5.14 (m, 1H), 3.93 (dt, *J* = 1.8, 10.2 Hz, 1H), 3.66 (m, 1H), 3.06 (dt, *J* = 2.3, 5.4 Hz, 1H), 2.97 (m, 1H), 2.37 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 132.8, 117.7, 61.5, 57.8, 54.7, 35.6; $[\alpha]_D^{25}$ 26 (*c* = 0.99, $CHCl_3$).



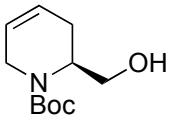
***tert*-Butyl Allyl((2*S*,3*S*)-1,2-dihydroxyhex-5-en-3-yl)carbamate (34).** ((2*R*,3*R*)-3-Allyloxiran-2-yl)methanol (4.4 g, 38.5 mmol) was dissolved in CH_2Cl_2 (150 mL). To the stirring solution, titanium tetraisoproxide (23.0 mL, 77.1 mmol) and

allylamine (3.6 mL, 46.2 mmol) were added in sequence. The mixture was stirred at reflux for 6 days. Upon consumption of the starting material, the reaction was quenched with a solution of 10% NaOH in brine, and stirred at room temperature for 12 h. The mixture was then filtered through a pad of Celite, washed with 10% HCl (30 mL); the aqueous phase was collected and basified with NaOH until pH = 8 - 9. The aqueous layer was extracted with CH_2Cl_2 (20 mL × 3) and the organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude material (2.9 g) was dissolved in MeOH (20 mL), basified with sodium bicarbonate (4.3 g, 115.5 mmol) and then di-*tert*-butyl dicarbonate (4.7 mL, 46.2 mmol) was added. The reaction vessel was then placed in an ultra-sonic cleaner and was allowed to react overnight. After the starting material was consumed, the mixture was filtered, washed with saturated NH_4Cl (20 mL), and extracted with EtOAc (20 mL × 3). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 50% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a light yellow oil was obtained (33% over two steps). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.82 - 5.70 (m, 2H), 5.15 (m, 2H), 5.07 (t, *J* = 8.4 Hz, 2H), 3.80 (m, 1H), 3.70 (d, *J* = 6.2 Hz, 2H), 3.57 (m, 2H), 3.03 (m, 1H), 2.66 (m, 1H), 2.46 (m, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 157.3, 135.4, 135.0, 117.2, 117.0, 80.9, 80.8, 73.5, 63.2, 58.2, 32.0, 28.4; ESI-HRMS: calc'd *m/e* for [M+Na^+] $C_{14}H_{25}NNaO_4$: 294.1681, found 294.1647; IR (neat, NaCl, cm^{-1}): 3409, 3078, 2878, 2931, 1667, 1457, 1407, 1367, 1332, 1250, 1174, 1152, 1080, 995, 960,

914, 861, 831, 776; $[\alpha]_D^{25}$ -2.2 (*c* = 0.41, $CHCl_3$).

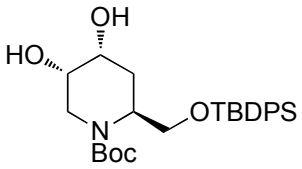


(*S*)-*tert*-Butyl 6-((*S*)-1,2-Dihydroxyethyl)-5,6-dihydropyridine-1(2*H*)-carboxylate (35). To a solution of diene **34** (1.6 g, 5.9 mmol) in CH_2Cl_2 (60 mL), the second generation of Grubbs catalyst (500 mg, 10 mol%) was added. After stirring at room temperature for 12 h, the solvent was removed *in vacuo*, and the crude residue was purified by flash column chromatography on silica gel, using 75% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (74%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.81 (t, *J* = 6.2 Hz, 1H,), 5.63 (dd, *J* = 2.6, 7.5 Hz, 1H), 4.14 (m, 2H), 3.64 - 3.52 (br s, 2H), 3.50 (m, 1H), 3.44 (d, *J* = 11.6 Hz, 2H), 2.62 (dd, *J* = 5.0, 17.8 Hz, 1H), 2.37 (m, 2H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 157.2, 123.4, 122.1, 81.0, 69.7, 62.5, 48.5, 41.4, 28.4, 24.4; ESI-HRMS: calc'd *m/e* for $[M+Na^+]$ $C_{12}H_{21}NNaO_4$: 266.1368, found 266.1360; IR (neat, NaCl, cm^{-1}): 3403, 2976, 2929, 1693, 1674, 1659, 1478, 1455, 1413, 1391, 1365, 1310, 1253, 1172, 1113, 1059, 988, 961, 925, 893, 857, 772, 657; $[\alpha]_D^{25}$ 30 (*c* = 1.1, $CHCl_3$).



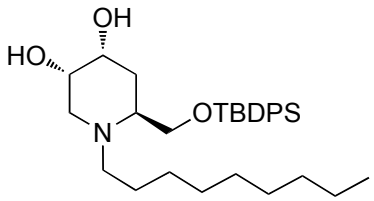
(*S*)-*tert*-Butyl 6-(Hydroxymethyl)-5,6-dihydropyridine-1(2*H*)-carboxylate (36). The diol **35** (781.0 mg, 3.2 mmol) was dissolved in a mixture of THF and H_2O (13

mL, 1:3, v/v). To the stirring solution, sodium periodate (1.0 g, 4.8 mmol) was added in portions over 30 min and then stirred at room temperature for 2 h. The reaction mixture was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (20 mL × 3). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resultant residue was dissolved in anhydrous ethanol (15 mL), and cooled to 0 °C. To the stirring solution, sodium borohydride (182.0 mg, 4.8 mmol) was added and the reaction was monitored by TLC. Upon consumption of the starting material, the solvent was removed *in vacuo*, and the residue was dissolved with CH_2Cl_2 and washed with H_2O. The aqueous layer was extracted with CH_2Cl_2 (20 mL × 2); the organic layers were combined, dried over $MgSO_4$, filtered and concentrated. The crude material was purified by flash column chromatography on silica gel, using 50% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (60%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.72 (m, 1H), 5.65 (m, 1H), 4.48 (m, 1H), 4.18 (m, 1H), 3.62 (dd, *J* = 1.8, 10.7 Hz, 2H), 3.56 (m, 1H), 2.40 (m, 1H), 2.05 (m, 1H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 154.1, 123.3, 122.6, 81.0, 63.0, 52.6, 40.7, 28.5, 25.2; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{11}H_{19}NNaO_3$: 236.1263, found 236.1213; IR (neat, NaCl, cm^{-1}): 3435, 2976, 2929, 1695, 1677, 1476, 1415, 1365, 1248, 1171, 1115, 1047, 958, 884, 858, 771; $[\alpha]_D^{25}$ 19 (*c* = 1.2, $CHCl_3$).



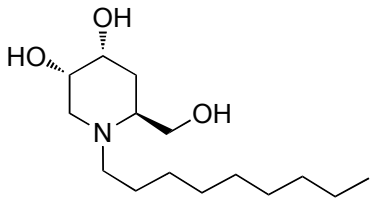
(2*S*,4*R*,5*S*)-*tert*-Butyl 2-((*tert*-Butyldiphenylsilyloxy)methyl)-4,5-dihydroxypiperidine-1-carboxylate (37). To a solution of compound **36** (478.0 mg, 2.2 mmol) in CH_2Cl_2 and DMF (20 mL, 1:1, v/v), *tert*-butyldiphenylsilyl chloride (1.7 mL, 6.7 mmol) and imidazole (305.0 mg, 4.5 mmol) were added in sequence at 0 °C. The mixture was then allowed to warm to room temperature and continued stirring for 2 hours. Upon completion, the reaction was diluted with H_2O (10 mL), and extracted with CH_2Cl_2 (20 mL × 2). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (7.5 mL) and cooled to 0 °C and a solution of *N*-methylmorpholine *N*-oxide (300.0 mg, 6.7 mmol) in H_2O (0.6 mL) was added. To the stirring solution, OsO_4 (0.42 mL, 2.5 wt%, 5 mol%) was added and the reaction was allowed to warm to room temperature and continued stirring for 12 h. Upon consumption of the starting material, the reaction was diluted with saturated ammonium chloride and extracted with CH_2Cl_2 (20 mL × 2). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 75% EtOAc:hexanes (v/v) as the eluent. After chromatographic purification, a colorless oil was obtained (60%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.64 (m, 4H), 7.41 (m, 6H), 4.44 (m, 1H), 4.19 (d, *J* = 13.8 Hz, 1H), 3.82 (m, 1H), 3.77 (m, 1H), 3.65 (dd, *J* = 2.6, 6.2 Hz, 2H), 2.93 (d, *J* = 14.4 Hz, 1H),

1.87 (dd, *J* = 5.4, 11.6 Hz, 2H), 1.43 (s, 9H), 1.05 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 135.58, 135.56, 133.14, 133.07, 129.8, 127.80, 127.79, 80.1, 67.6, 66.5, 63.1, 45.0, 28.3, 28.2, 26.8, 19.1; ESI-HRMS: calc'd *m/e* for $[M+Na^+]$ $C_{27}H_{39}NNaO_5Si$: 508.2495, found 508.2494; IR (neat, NaCl, cm^{-1}): 3415, 2961, 2931, 2858, 1473, 1428, 1391, 1365, 1248, 1173, 1137, 1113, 1077, 1022, 824, 742, 702, 611; $[\alpha]_D^{25}$ -11 (*c* = 0.98, $CHCl_3$).



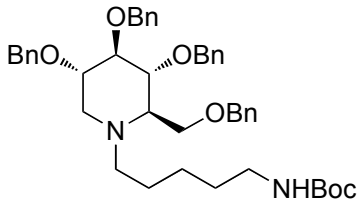
(3*S*,4*R*,6*S*)-6-((*tert*-Butyldiphenylsilyloxy)methyl)-1-nonylpiperidine-3,4-diol (38). The diol derivative **37** (119.0 mg, 0.2 mmol) was dissolved in formic acid (10 mL per 1 mmol of starting material). The mixture was allowed to stir for 3 h at room temperature. Upon consumption of the starting material, the solvent was removed *in vacuo*, and the remaining residue was left on high vacuum for 1 h. The residue was then charged with dichloroethane (15 mL). To the stirring suspension, triethylamine (30.0 μL, 0.2 mmol) and nonyl aldehyde (67.0 μL, 0.4 mmol) were added in sequence. The mixture was allowed to stir for 15 min until it turned into a clear solution. Next, sodium triacetoxyborohydride (109.7 mg, 0.5 mmol) was added to the reaction. The pH value of the reaction was adjusted to be slightly acidic by acetic acid. The mixture was allowed to react at room temperature overnight. The reaction was then quenched with saturated sodium bicarbonate and extracted with CH_2Cl_2 (2 x 20 mL). The organic layers were

combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 4% $MeOH/CH_2Cl_2$ + 1% NH_4OH as the eluent. After chromatographic purification, a colorless oil was obtained (67%). ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.64 (d, *J* = 7.6 Hz, 4H), 7.42 (m, 6H), 4.05 - 3.80 (br s, 2H), 3.67 (d, *J* = 8.9 Hz, 1H), 3.11 (m, 2H), 2.86 (m, 4H), 1.93 (s, 2H), 1.55 (br, 2H), 1.24 (m, 12H), 1.06 (s, 9H), 0.88 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 135.5, 130.4, 128.0, 126.7, 82.3, 77.7, 69.7, 67.1, 55.7, 51.0, 43.4, 34.9, 32.4, 31.8, 30.5, 29.7, 29.4, 29.1, 26.8, 22.6, 14.4; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{31}H_{50}NO_3Si$: 512.3560, found 512.3592; IR (neat, NaCl, cm^{-1}): 3369, 3046, 3044, 2927, 2856, 1638, 1588, 1550, 1465, 1428, 1391, 1361, 1261, 1111, 1082, 823, 801, 740, 702; $[\alpha]_D^{25}$ -2.7 (*c* = 0.44, $CHCl_3$).



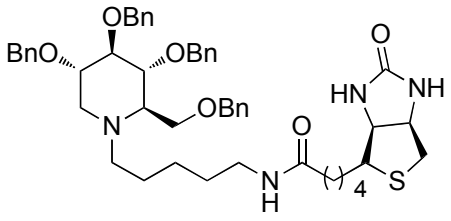
(3*S*,4*R*,6*S*)-6-(Hydroxymethyl)-1-nonylpiperidine-3,4-diol (39). The silyl ether **38** (81.0 mg, 0.2 mmol) was dissolved in THF (10 mL) and cooled to 0 °C. To the stirring solution, 1M tetrabutylammonium fluoride (0.18 mL, 0.2 mmol) was added slowly. The mixture was allowed to react for 1 h at 0 °C. Upon completion of the reaction, the solvent was removed *in vacuo*, and the crude material was subjected to chromatography with 15% $MeOH/CH_2Cl_2$ as the eluent. The resultant relatively pure product was contaminated with the residual

tetrabutylammonium fluoride. It was then loaded on a Dowex resin (acidic form) column and eluted with H_2O. Tubes containing the product were collected and the solvent was removed *in vacuo* to yield the desired product as a colorless oil (96%). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 3.97 (dd, *J* = 3.5, 6.7 Hz, 1H), 3.67 (m, 1H), 3.61 (t, *J* = 3.7 Hz, 2H), 2.81 (m, 1H), 2.77 (dd, *J* = 4.4, 6.2 Hz, 2H), 2.62 (t, *J* = 10.1 Hz, 2H), 1.90 (m, 1H), 1.68 (m, 1H), 1.56 (m, 2H), 1.32 (m, 12H), 0.92 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 79.5, 73.9, 69.0, 64.4, 57.9, 50.2, 44.1, 33.5, 33.0, 30.7, 30.6, 30.5, 30.4, 23.7, 14.4; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{15}H_{32}NO_3$: 274.2382, found 274.2384; $[\alpha]_D^{25}$ -18 (*c* = 0.56, MeOH).



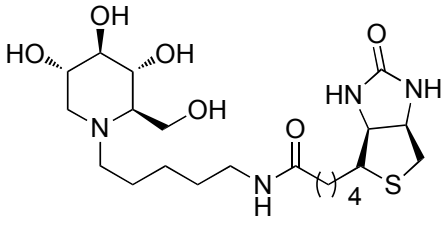
***tert*-Butyl (5-((2*R*,3*R*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)pentyl)carbamate (40).** (2*R*,3*R*,4*S*)-2,3,4,6-tetrakis(benzyloxy)-5-oxohexanal (431.0 mg, 0.8 mmol) obtained from **1** by oxidation (see synthesis of compound **2**) was dissolved in MeOH (5 mL 4Å molecular sieves (300 mg) were added. A solution of Boc-protected-1,5-pentadiamine (0.5 mL, 2.4 mmol) in MeOH was then added to the reaction. Next, sodium cyanoborohydride (140.0 mg, 2.1 mmol) was added, and the pH of the reaction was kept < 7 by adding acetic acid. The reaction was stirred at 50 °C overnight, and quenched with 1 M NaOH (5 mL). The reaction mixture

was filtered through a pad of Celite. The filtrated was diluted with H_2O (40 mL) and extracted with CH_2Cl_2. The filtrate was dried over MgSO4 and the solvent was removed under reduced pressure. The crude products was purified by flash column chromatography on silica gel, using 60% EtOAc:hexanes (v/v) as the eluent. After purification, a colorless oil was obtained (52%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.32 - 7.28 (m, 18H), 7.14 (d, *J* = 6.5 Hz, 2H), 4.95 (d, *J* = 11.1 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.81 (d, *J* = 11.1 Hz, 1H), 4.69 (d, *J* = 11.5 Hz, 1H), 4.64 (d, *J* = 11.5 Hz, 1H), 4.49 (d, *J* = 12.3 Hz, 1H), 4.45 (m, 1H), 4.41 (d, *J* = 10.8 Hz, 1H), 3.64 (m, 2H), 3.58 (t, *J* = 9.3 Hz, 1H), 3.52 (d, *J* = 10.2 Hz, 1H), 3.45 (t, *J* = 9.1 Hz, 1H), 3.06 (dd, *J* = 4.8, 11.1 Hz, 3H), 2.65 (m, 1H), 2.54 (m, 1H), 2.28 (d, *J* = 9.5 Hz, 1H), 2.20 (t, *J* = 10.8 Hz, 1H), 1.45 (s, 9H), 1.39 (t, *J* = 7.4 Hz, 2H), 1.32 - 1.09 (m, 5H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 155.9 139.0, 138.5, 137.8, 128.5, 128.37, 128.33, 128.31, 127.86, 127.84, 127.6, 127.5, 127.4, 87.4, 78.6, 78.5, 75.3, 75.2, 73.4, 72.8, 65.2, 63.7, 54.4, 52.1, 40.5, 30.3, 29.8, 29.7, 28.5, 24.6, 23.3.



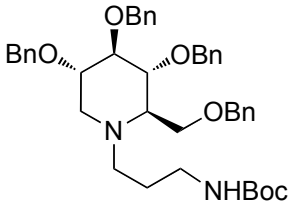
5-((3a*S*,4*R*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-*N*-(5-((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)pentyl)pentanamide (41). The carbamate **40** (164.6 mg, 0.2 mmol) was placed in a flame-dried flask and cooled to 0 °C. To the flask was added 4M HCl

in dioxane (4.0 mL, 16.0 mmol). The solvent was removed under reduced pressure after the reaction was completed. The crude material was dissolved in anhydrous dimethylformamide (1 mL) and *N*-methylmorpholine (0.2 mL, 1.8 mmol) was added. To the stirring solution, a solution of biotin (60 mg, 1.0 equiv) in DMF (3 mL) was added, followed by the addition of the coupling reagent *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate(HATU, 90 mg, 0.2 mmol). The mixture was allowed to stir at room temperature for 2 days. The mixture was then washed with H_2O and the aqueous phase was extracted with EtOAc (20 mL × 2). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography on silica gel, using 10% MeOH/CH_2Cl_2 + 1% NH_4OH as the eluent. After purification, a yellow oil was obtained (92%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.32 - 7.18 (m, 20H), 6.45 (s, 1H), 5.86 (s, 1H), 5.32 - 4.15 (m, 10H), 3.96 - 3.62 (m, 5H), 3.17 - 3.05 (m, 6H), 2.75 - 2.52 (m, 3H), 2.17 (s, 2H), 1.63 - 1.29 (m, 14H).



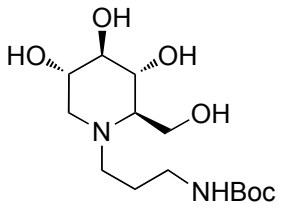
5-((3a*S*,4*R*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-*N*-(5-((2*R*,3*R*,4*R*,5*S*)-3,4,5-trihydroxy-2-(hydroxymethyl)piperidin-1-yl)pentyl)pentanamide (42). The benzylated compound **41** (400 mg, 0.5 mmol) was dissolved in a mixture of ethanol and H_2O (10 mL, 1:1, v/v). To the stirring

solution, palladium chloride (348.0 mg, 2.1 mmol) was added carefully. The mixture was allowed to stir at room temperature under hydrogen gas (1 atmosphere) for 2 days. Upon completion of the reaction, the catalyst was filtered off by passing the reaction mixture through a Celite pad. The solvent was then removed *in vacuo* and the crude product was purified by flash column chromatography on silica gel, using 10% MeOH/CH_2Cl_2 + 1% NH_4OH as the eluent. After purification, an amorphous solid (31%) was obtained. ^1H NMR (D_2O, 400 MHz, ppm): δ 7.80 - 7.65 (br s, 1H), 4.96 (m, 1H), 3.77 (m, 1H), 4.19 (m, 4H), 4.01 (m, 2H), 3.72 (m, 2H), 3.55 (m, 1H), 2.38 (m, 1H), 3.12 (m, 1H), 2.95 (m, 1H), 2.62 (m, 4H), 2.27 (m, 2H), 1.94 (m, 5H), 1.66 (m, 5H); ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{21}H_{39}N_4O_6S$: 475.2590, found 475.2597.



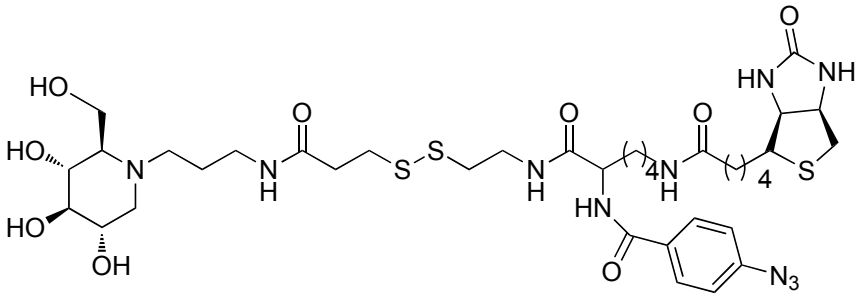
***tert*-Butyl (3-((2*R*,3*R*,4*R*,5*S*)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)propyl)carbamate (43).** (2*R*,3*R*,4*S*)-2,3,4,6-tetrakis(benzyloxy)-5-oxohexanal (60.3 mg, 0.1 mmol) generated from the Swern oxidation was dissolved in MeOH (5 mL) and 3Å molecular sieves (50.0 mg) were added. A solution of the Boc-protected-1,3-propyldiamine HCl (71.5 mg, 0.3 mmol) and triethylamine (46 μL, 0.3 mmol) in MeOH were then added to the reaction in sequence. Next, sodium cyanoborohydride (18.2 mg, 0.3 mmol) was added, and the pH of the reaction was kept < 7 by adding acetic acid. The

reaction was stirred at 50 °C overnight, and quenched with 1 M NaOH (5 mL). The reaction mixture was filtered through a pad of Celite. The filtrated was diluted with H_2O (40 mL) and extracted with CH_2Cl_2. The organic layers were combined and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude residue was purified by flash column chromatography on silica gel, using 60% EtOAc:hexanes (v/v) as the eluent. After purification, a colorless oil was obtained (58%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.32 - 7.28 (m, 18H), 7.10 (dd, *J* = 2.5, 7.7 Hz, 2H), 5.26 - 5.20 (br s, 1H), 4.96 (d, *J* = 11.1 Hz, 1H), 4.84 (d, *J* = 10.8 Hz, 1H), 4.80 (d, *J* = 11.1 Hz, 1H), 4.70 (d, *J* = 11.6 Hz, 1H), 4.63 (d, *J* = 11.5 Hz, 1H,), 4.54 (d, *J* = 12.2 Hz, 1H), 4.45 (d, *J* = 12.2 Hz, 1H), 4.33 (d, *J* = 10.8 Hz, 1H), 3.66 (dd, *J* = 2.1, 10.4 Hz, 1H), 3.60 (d, *J* = 2.3 Hz, 1H), 3.57 (d, *J* = 3.3 Hz, 1H), 3.54 (d, *J* = 9.3 Hz, 1H), 3.46 (t, *J* = 8.9 Hz, 1H), 3.16 (m, 1H), 3.04 (dd, *J* = 4.8, 11.2 Hz, 1H), 2.98 (m, 1H), 2.81 (m, 1H), 2.34 (m, 1H), 2.24 (d, *J* = 9.3 Hz, 1H), 2.02 (m, 1H), 1.42 (s, 9H), 1.25 (t, *J* = 7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 156.2 139.0, 138.5, 128.4, 128.3, 128.0, 127.9, 127.84, 127.80, 127.7, 127.6, 127.5, 127.4, 87.3, 78.6, 75.3, 75.2, 74.6, 73.3, 72.9, 65.4, 64.7, 58.8, 53.8, 39.0, 28.5, 24.9, 22.7.



***tert*-Butyl (3-((2*R*,3*R*,4*R*,5*S*)-3,4,5-Trihydroxy-2-(hydroxymethyl)piperidin-1-yl)propyl)carbamate (44).** A mixture of **43** (43.0 mg, 0.06 mmol), ammonium

formate (80.0 mg, 0.6 mmol) and 10% palladium on carbon (45.0 mg, 0.3 g per mM of *O*-benzyl group) was refluxed in MeOH in a sealed reaction vessel. The reaction was monitored by TLC, and upon completion of the reaction, the catalyst was carefully filtered off by passing the mixture through a Celite pad. The solvent was subsequently removed *in vacuo*. The neutral residue was purified by flash column chromatography, using 10% MeOH/ CH_2Cl_2 + 1% NH_4OH (v/v) as the eluent. After purification, a colorless oil was obtained (77%). ^1H NMR (MeOD, 400 MHz, ppm): δ 3.97 (d, *J* = 12.2 Hz, 1H), 3.87 (d, *J* = 11.0 Hz, 1H), 3.58 (m, 1H), 3.47 (t, *J* = 9.5 Hz, 1H), 3.25 (m, 1H), 3.22 (t, *J* = 4.8 Hz, 1H), 3.15 (m, 1H), 3.09 (t, *J* = 6.7 Hz, 2H), 2.92 (m, 1H), 2.63 (d, *J* = 7.1 Hz, 1H), 2.59 (t, *J* = 11.2 Hz, 1H), 1.79 (m, 2H), 1.42 (s, 9H), 1.26 (s, 1H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 158.6, 80.2, 79.1, 70.2, 69.0, 67.5, 56.8, 56.0, 51.5, 38.9, 28.7, 25.4.

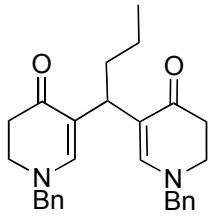


4-Azido-*N*-(5,13,20-trioxo-24-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-1-((2*R*,3*R*,4*R*,5*S*)-3,4,5-trihydroxy-2-(hydroxymethyl)piperidin-1-yl)-8,9-dithia-4,12,19-triazatetracosan-14-yl)benzamide (45). The aminosugar derivative **44** (3.5 mg, 0.01 mmol) was dissolved in formic acid (2 mL) and stirred for 1 h. The solvent was removed

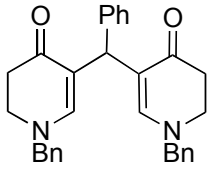
when starting material was completely consumed. The residue was then dissolved in DMSO (1 mL) and stirred in a reaction vessel that was wrapped in aluminum foil to exclude light. To the stirring mixture, Sulfo-SBED (10 mg, 0.01 mmol) was added to generate the photoaffinity label **45**. The sample was submitted for the biological assay in the form of a 0.01 M solution in DMSO, (95%). ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{37}H_{59}N_{10}O_9S_3$: 883.3629, found 883.3623.

3.3.2 Chapter 2

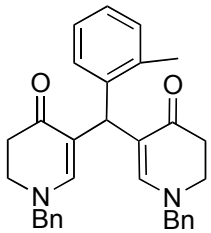
General Procedure for the Synthesis of bis-Addition Products of Enaminones with Aldehydes (Table 4): The enaminone was dissolved in THF or CH_2Cl_2 (5 mL per 0.1 mmol), and stirred at room temperature. To the stirring solution, trimethylsilyl chloride (1 equiv relative to the enaminone) was added, followed by the addition of the aldehyde (1 equiv). The reaction was monitored by TLC and quenched with saturated sodium bicarbonate when the starting material was consumed. The organic layer was separated and the aqueous phase was extracted with EtOAc (10 mL × 2). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 75% EtOAc:hexanes (v/v) as the eluent.



5,5'-(Butane-1,1-diyl)bis(1-benzyl-2,3-dihydropyridin-4(1*H*)-one). After chromatographic purification, a viscous yellow oil was obtained (86%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.51 (s, 2H), 7.37 - 7.26 (m, 10H), 4.33 (s, 4H), 3.28 (t, *J* = 7.8 Hz, 1H), 3.20 (t, *J* = 7.7 Hz, 4H), 2.38 (t, *J* = 8.0 Hz, 4H), 1.79 (dd, *J* = 7.6, 15.6 Hz, 2H), 1.26 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H); ESI-HRMS: calc'd *m/e* for [M+Na^+] $C_{28}H_{32}N_2NaO_2$: 451.2361, found 451.2386.

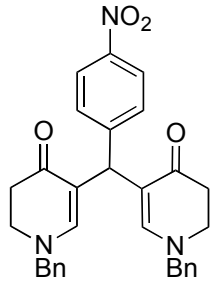


5,5'-(Phenylmethylene)bis(1-benzyl-2,3-dihydropyridin-4(1*H*)-one). After chromatographic purification, a viscous yellow oil was obtained (90%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 - 7.30 (m, 9H), 7.25 (s, 2H), 7.22 - 7.20 (m, 6H), 5.20 (s, 1H), 4.28 (s, 4H), 3.29 (t, *J* = 7.8 Hz, 4H), 2.46 (t, *J* = 8.3 Hz, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 199.9, 155.5, 136.1, 128.9, 128.3, 128.1, 127.9, 127.7, 125.5, 111.5, 60.2, 46.6, 41.5, 36.1; ESI-HRMS: calc'd *m/e* for [M+Na^+] $C_{31}H_{30}N_2NaO_2$: 485.2205, found 485.2234.



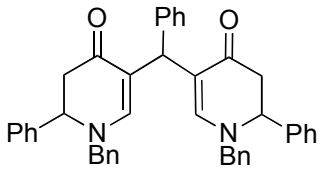
5,5'-(2-Tolylmethylene)bis(1-benzyl-2,3-dihydropyridin-4(1*H*)-one). After chromatographic purification, a yellow oil was obtained (83%). ^{1}H NMR ($CDCl_3$,

400 MHz, ppm): δ 7.35 - 7.26 (m, 7H), 7.20 - 7.08 (m, 8H), 6.88 (s, 2H), 5.15 (s, 1H), 4.22 (d, *J* = 2.9 Hz, 4H), 3.27 (t, *J* = 7.8 Hz, 4H), 2.46 (t, *J* = 3.6 Hz, 4H), 2.24 (s, 3H), 3.29 (t, *J* = 7.8 Hz, 4H), 2.46 (t, *J* = 8.3 Hz, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 189.3, 154.1, 142.1, 136.4, 136.1, 130.4, 128.8, 128.1, 127.6, 127.4, 125.8, 125.4, 110.7, 60.1, 46.8, 38.0, 36.1, 19.5; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{32}H_{32}N_2NaO_2$: 499.2361, found 499.2371.



5,5'-((4-Nitrophenyl)methylene)bis(1-benzyl-2,3-dihydropyridin-4(1*H*)-one).

After chromatographic purification, a yellow oil was obtained (75%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.10 (dd, *J* = 1.7, 7.0 Hz, 2H), 7.41 - 7.33 (m, 8H), 7.25 - 7.21 (m, 6H), 5.01 (s, 1H), 4.32 (s, 4H), 3.34 (t, *J* = 7.7 Hz, 4H), 2.47 (t, *J* = 8.1 Hz, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 189.1, 155.2, 152.7, 145.9, 135.8, 129.0, 128.8, 128.3, 127.8, 123.2, 110.4, 60.4, 60.2, 46.6, 41.8, 35.9; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{31}H_{29}N_3NaO_4$: 530.2056, found 530.2074.



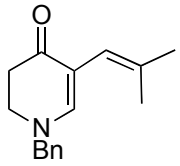
5,5'-(Phenylmethylene)bis(1-benzyl-2-phenyl-2,3-dihydropyridin-4(1*H*)-one).

After chromatographic purification, two isomers were obtained (1:2, 99%, combined yield). Isomer 1, yellow oil (Rf 0.38, 75% EtOAc/hexane): ^{1}H NMR ($CDCl_3$ 400 MHz, ppm): δ 7.34 - 7.23 (m, 23H), 7.02 (m, 4H), 5.03 (s, 1H), 4.41 (t, *J* = 7.3 Hz, 2H), 4.24 (d, *J* = 15.1 Hz, 2H), 4.03 (d, *J* = 15.1 Hz, 2H), 2.82 (dd, *J* = 6.8, 16.2 Hz, 2H), 2.70 (dd, *J* = 8.3, 16.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 188.3, 155.3, 143.6, 138.7, 136.2, 128.9, 128.7, 128.5, 128.03, 127.97, 127.95, 127.8, 127.2, 125.6, 111.7, 60.4, 57.5, 44.3, 41.8;

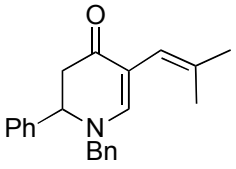
Isomer 2, yellow oil (Rf 0.25, 75% EtOAc/hexane): ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.34 - 7.18 (m, 23H), 7.05 - 6.99 (m, 4H), 5.04 (s, 1H), 4.43 (m, 2H), 4.25 (m, 2H), 4.03 (m, 2H), 2.81 (m, 2H), 2.69 (m, 2H); 3.83 (d, *J* = 13.5 Hz, 2H), 3.58 (dd, *J* = 3.7, 10.9 Hz, 2H), 3.22 (m, 2H), 2.93 (d, *J* = 13.5 Hz, 2H), 2.66 (m, 4H), 2.54 (m, 2H), 2.38 (m, 1H), 2.33 (m, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 188.7, 188.3, 188.1, 155.5, 155.2, 155.1, 143.7, 138.74, 138.68, 136.3, 136.19, 136.17, 128.9, 128.7, 128.5, 128.3, 128.1, 128.0, 127.99, 127.95, 127.85, 127.83, 127.29, 127.25, 125.6, 125.5, 112.1, 111.7, 111.1, 60.7, 60.6, 60.4, 57.6, 57.5, 57.4, 44.6, 44.4, 44.2, 41.8, 41.5;

General Procedure for the Synthesis of β-Elimination Products: The enaminone was dissolved in CH_2Cl_2 (10 mL per 0.1 mmol), and stirred at room temperature. To the stirring solution, trimethylsilyl chloride (1 equiv relative to the enaminone) was added, followed by the addition of isobutylaldehyde and Ti(OiPr)4 (1 equiv). The reaction vessel was then sealed, heated to 60 °C and monitored by TLC and quenched with saturated sodium bicarbonate when the

starting material was completely consumed. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (10 mL × 2). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 50% EtOAc:hexanes (v/v) as the eluent.



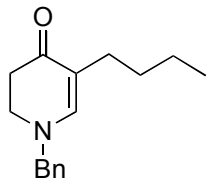
1-Benzyl-5-(2-methylprop-1-en-1-yl)-2,3-dihydropyridin-4(1*H*)-one. After chromatographic purification, a yellow oil was obtained (44%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.41 - 7.34 (m, 3H), 7.28 (d, *J* = 1.9 Hz, 2H), 7.14 (s, 1H), 5.90 (s, 1H), 4.39 (s, 2H), 3.38 (t, *J* = 7.6 Hz, 2H), 2.48 (t, *J* = 7.9 Hz, 2H), 1.81 (d, *J* = 0.8 Hz, 3H), 1.71 (d, *J* = 0.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 153.6, 118.4, 110.8, 60.2, 46.7, 35.9, 26.3, 19.7; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{16}H_{20}NO$: 242.1545, found 242.1550.



1-Benzyl-5-(2-methylprop-1-en-1-yl)-2-phenyl-2,3-dihydropyridin-4(1*H*)-one. After chromatographic purification, a yellow oil was obtained (77%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 - 7.25 (m, 9H), 7.16 - 7.14 (m, 2H), 5.90 (s, 1H), 4.51 (t, *J* = 7.5 Hz, 1H), 4.37 (d, *J* = 15.0 Hz, 1H), 4.17 (d, *J* = 15.0 Hz, 1H), 2.87

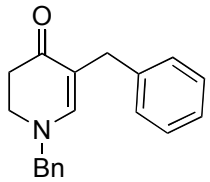
(dd, *J* = 6.9, 16.4 Hz, 1H), 2.73 (dd, *J* = 8.0, 16.4 Hz, 1H), 1.82 (s, 3H), 1.73 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 189.1, 153.2, 138.6, 136.1, 132.3, 129.0, 128.9, 128.3, 128.2, 127.8, 127.1, 118.2, 109.0, 60.6, 57.5, 43.8, 26.3, 19.8; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{22}H_{24}NO$: 318.1858, found 318.0979.

General Procedure for the Synthesis of Alkyl Products 46 (Table 5): The enaminone was dissolved in CH_2Cl_2 (10 mL per 0.1 mmol), and stirred at room temperature. To the stirring solution, trifluoroacetic acid (4 equiv relative to the enaminone) was added, followed by the addition of triethylsilane and the aldehyde (1 equiv, respectively). The reaction was heated to 60 °C in a sealed vessel and monitored by TLC. The reaction was quenched with saturated sodium bicarbonate when all starting material was consumed. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (10 mL × 2). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 20% EtOAc:hexanes (v/v) as the eluent.

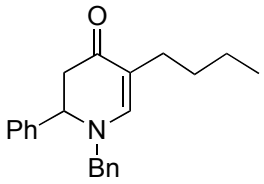


1-Benzyl-5-butyl-2,3-dihydropyridin-4(1*H*)-one (46a). After chromatographic purification, a yellow oil was obtained (81%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.33 - 7.25 (m, 3H), 7.19 (d, *J* = 6.9 Hz, 2H), 6.94 (s, 1H), 4.23 (s, 2H), 3.21 (t, *J*

= 7.7 Hz, 2H), 2.37 (t, *J* = 7.6 Hz, 2H), 2.05 (t, *J* = 6.8 Hz, 2H), 1.33 - 1.21 (m, 4H), 0.83 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 189.8, 151.5, 135.3, 127.9, 127.7, 127.1, 126.9, 126.6, 109.8, 58.9, 46.1, 35.1, 31.0, 26.1, 21.4, 13.0; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{16}H_{22}NO$: 244.1701, found 244.1704.

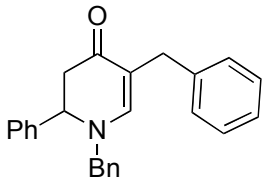


1,5-Dibenzyl-2,3-dihydropyridin-4(1*H*)-one (46b). After chromatographic purification, a yellow oil was obtained (85%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 - 7.15 (m, 10H), 6.90 (s, 1H), 4.25 (s, 2H), 3.50 (s, 2H), 3.30 (t, *J* = 7.8 Hz, 2H), 2.48 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 190.1, 153.4, 141.6, 136.0, 128.9, 128.7, 128.3, 128.2, 127.6, 125.7, 110.1, 59.9, 46.9, 35.8, 32.8; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{19}H_{20}NO$: 278.1545, found 278.1520.

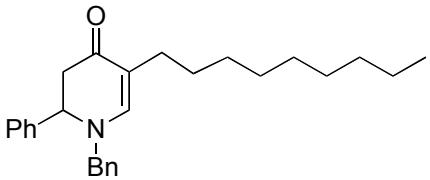


1-Benzyl-5-butyl-2-phenyl-2,3-dihydropyridin-4(1*H*)-one (46c). After chromatographic purification, a yellow oil was obtained (92%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.35 - 7.30 (m, 6H), 7.25 - 7.22 (m, 2H), 7.14 (s, 1H), 7.11 (m, 2H), 4.42 (t, *J* = 8.1 Hz, 1H), 4.29 (d, *J* = 15.2 Hz, 1H), 4.06 (d, *J* = 15.2 Hz, 1H),

2.73 (m, 2H), 2.16 (m, 2H), 1.42 (m, 2H), 1.34 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 189.8, 152.4, 139.0, 136.4, 128.9, 128.8, 128.2, 128.0, 127.7, 127.2, 110.0, 61.2, 57.0, 44.2, 32.1, 27.0, 22.4, 14.0; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{22}H_{26}NO$: 320.2014, found 320.1999.

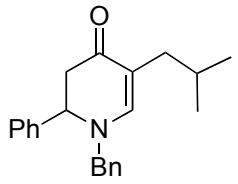


1,5-Dibenzyl-2-phenyl-2,3-dihydropyridin-4(1*H*)-one (46d). After chromatographic purification, a yellow oil was obtained (86%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.34 - 7.17 (m, 13H), 7.03 (s, 2H), 7.01 (s, 1H), 4.43 (dd, *J* = 2.2, 9.0 Hz, 1H), 4.22 (d, *J* = 15.1 Hz, 1H), 4.03 (d, *J* = 15.1 Hz, 1H), 3.57 (d, *J* = 15.7 Hz, 1H), 3.52 (d, *J* = 15.0 Hz, 1H), 2.82 (dd, *J* = 6.8, 16.4 Hz, 1H), 2.73 (dd, *J* = 9.2, 16.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 189.1, 153.5, 141.5, 138.7, 136.0, 129.0, 128.8, 128.7, 128.3, 128.2, 128.1, 127.7, 127.2, 125.8, 110.5, 61.1, 57.2, 44.0, 32.7; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{25}H_{24}NO$: 354.1858, found 354.1821.

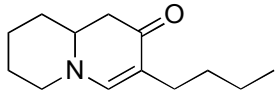


1-Benzyl-5-nonyl-2-phenyl-2,3-dihydropyridin-4(1*H*)-one (46e). After chromatographic purification, a yellow oil was obtained (77%). ^{1}H NMR ($CDCl_3$,

400 MHz, ppm): δ 7.33 - 7.11 (m, 11H), 4.42 (t, *J* = 7.8 Hz, 1H), 4.30 (d, *J* = 15.1 Hz, 1H), 4.06 (d, *J* = 15.0 Hz, 1H), 2.73 (m, 2H), 2.15 (d, *J* = 5.3 Hz, 2H), 1.42 (s, 2H), 1.27 (s, 14H), 0.89 (t, *J* = 6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 189.8, 152.5, 139.0, 136.4, 128.9, 128.8, 128.2, 128.0, 127.7, 127.2, 111.0, 61.2, 57.0, 44.2, 31.9, 29.9, 29.7, 29.6, 29.4, 29.37, 27.3, 22.7, 14.1; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{27}H_{36}NO$: 390.2797, found 390.2776.

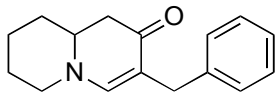


1-Benzyl-5-isobutyl-2-phenyl-2,3-dihydropyridin-4(1*H*)-one (46f). After chromatographic purification, a yellow oil was obtained (93%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.36 - 7.30 (m, 6H), 7.25 - 7.22 (m, 2H), 7.12 (m, 2H), 7.11 (s, 1H), 4.43 (dd, *J* = 2.0, 8.9 Hz, 1H), 4.31 (d, *J* = 15.1 Hz, 1H), 4.08 (d, *J* = 15.2 Hz, 1H), 2.78 (dd, *J* = 6.8, 16.4 Hz, 1H), 2.69 (dd, *J* = 9.1, 16.4 Hz, 1H), 2.01 (m, 2H), 1.76 (m, 1H), 0.89 (d, *J* = 3.9 Hz, 3H), 0.87 (d, *J* = 3.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 189.8, 153.1, 138.9, 136.4, 128.9, 128.8, 128.1, 128.0, 127.7, 127.2, 109.9, 61.1, 57.1, 44.2, 36.7, 28.3, 22.4, 22.3; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{22}H_{26}NO$: 320.2014, found 320.2013.

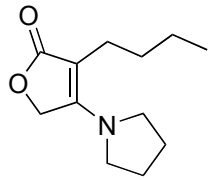


3-Butyl-7,8,9,9a-tetrahydro-1*H*-quinolizin-2(6*H*)-one (46g). After

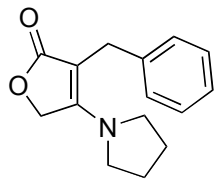
chromatographic purification, a colorless oil was obtained (88%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.64 (s, 1H), 3.26 (dt, *J* = 2.0, 12.4 Hz, 1H), 3.10 (m, 1H), 2.80 (dt, *J* = 2.9, 12.6 Hz, 1H), 2.36 (dd, *J* = 5.2, 16.2 Hz, 1H), 2.26 (dd, *J* = 13.8, 16.2 Hz, 1H), 2.02 (m, 2H), 1.77 (m, 1H), 1.67 (m, 2H), 1.51 (dddt, *J* = 4.2, 12.8, 12.8, 12.8 Hz, 1H), 1.39 (m, 1H), 1.32 - 1.17 (m, 5H), 0.81 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 192.0, 153.2, 112.1, 57.7, 52.8, 43.7, 32.1, 31.6, 26.8, 25.6, 23.2, 22.4, 14.0; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{13}H_{22}NO$: 208.1701, found 208.1711.



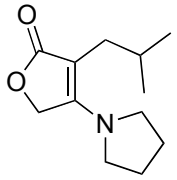
3-Benzyl-7,8,9,9a-tetrahydro-1*H*-quinolizin-2(6*H*)-one (46h). After chromatographic purification, a colorless oil was obtained (93%). 1H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.20 - 7.06 (m, 5H), 6.54 (s, 1H), 3.42 (d, *J* = 15.3 Hz, 1H), 3.36 (d, *J* = 15.3 Hz, 1H), 3.21 - 3.11 (m, 2H), 2.79 (dt, *J* = 2.9, 12.6 Hz, 1H), 2.42 (dd, *J* = 5.2, 16.3 Hz, 1H), 2.29 (dd, *J* = 13.8, 16.3 Hz, 1H), 1.77 - 1.61 (m, 3H), 1.52 - 1.18 (m, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 191.3, 154.1, 141.6, 128.7, 128.3, 125.7, 111.2, 57.5, 52.9, 43.4, 32.6, 31.7, 25.6, 23.1; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{16}H_{19}NNaO$: 264.1364, found 264.1369.



3-Butyl-4-(pyrrolidin-1-yl)furan-2(5*H*)-one (46i). After chromatographic purification, a colorless oil was obtained (89%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.53 (s, 2H), 3.44 (s, 4H), 2.30 (t, *J* = 7.2 Hz, 2H), 1.97 (t, *J* = 6.7 Hz, 4H), 1.41 (m, 2H), 1.34 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 176.1, 158.9, 92.5, 65.4, 47.3, 33.1, 24.2, 22.1, 21.6, 13.0; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{12}H_{19}NNaO_2$: 232.1313, found 232.1299.

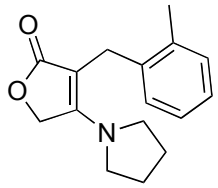


3-Benzyl-4-(pyrrolidin-1-yl)furan-2(5*H*)-one (46j). After chromatographic purification, a colorless oil was obtained (98%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.21 - 7.09 (m, 5H), 4.57 (s, 2H), 3.67 (s, 2H), 3.33 (s, 4H), 1.83 (t, *J* = 6.6 Hz, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 177.4, 161.5, 141.9, 128.6, 128.5, 128.2, 127.8, 125.9, 66.6, 48.5, 28.6, 25.1; ESI-HRMS: calc'd *m/e* is [M+Na$^+$] $C_{15}H_{17}NNaO_2$: 266.1157, found 266.1156.

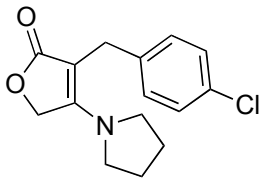


3-Isobutyl-4-(pyrrolidin-1-yl)furan-2(5*H*)-one (46k). After chromatographic purification, a colorless oil was obtained (88%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.57 (s, 2H), 3.67 (s, 2H), 3.43 (s, 4H), 2.19 (d, *J* = 7.3 Hz, 2H), 1.97 (m,

4H), 1.76 (m, 1H), 0.90 (s, 3H), 0.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 177.4, 160.5, 66.5, 48.5, 32.1, 30.1, 27.3, 25.2, 22.3; ESI-HRMS: calc'd [*m/e*] is [M+Na^+] $C_{12}H_{19}NNaO_2$ 232.1313, found 232.1310.



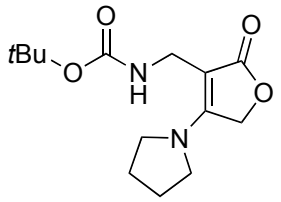
3-(2-Methylbenzyl)-4-(pyrrolidin-1-yl)furan-2(5*H*)-one (46l). After chromatographic purification, a colorless oil was obtained (97%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.14 - 7.08 (m, 3H), 6.97 (d, *J* = 7.4 Hz, 1H), 4.71 (s, 2H), 3.65 (s, 2H), 3.35 (t, *J* = 6.4 Hz, 4H), 2.32 (s, 3H), 1.86 (t, *J* = 6.6 Hz, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 177.6, 162.1, 139.2, 135.5, 129.9, 126.9, 126.1, 125.9, 66.7, 48.1, 26.0, 25.1, 19.7; ESI-HRMS: calc'd *m/e* for [M+Na^+] $C_{16}H_{19}NNaO_2$: 280.1313, found 280.1302.



3-(4-Chlorobenzyl)-4-(pyrrolidin-1-yl)furan-2(5*H*)-one (46m). After chromatographic purification, a colorless oil was obtained (96%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.23 (t, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.64 (s, 2H), 3.69 (s, 2H), 3.39 (s, 4H), 1.91 (m, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 176.5, 160.5, 139.4, 130.6, 128.1, 127.6, 65.7, 47.5, 27.1, 24.1; ESI-

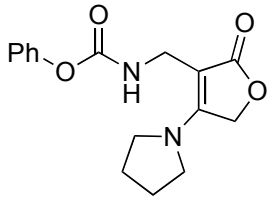
HRMS: calc'd *m/e* for [M+Na^+] $C_{15}H_{16}ClNNaO_2$: 300.0767, found 300.0742.

General Procedure for the Synthesis of Aminomethylation Products 47 (Table 6): The enaminone was dissolved in dichloroethane (10 mL per 0.1 mmol), and stirred at room temperature. To the stirring solution, lithium perchlorate (1.2 equiv relative to the enaminone) was added, followed by the addition of the carbamate (2.0 equiv) and paraformaldehyde (2.0 equiv) in sequence. The reaction was heated to 90 °C in a sealed vessel and monitored by TLC and quenched with saturated sodium bicarbonate when the starting material was completely consumed. The organic layer was separated and the aqueous phase was extracted with CH_2Cl_2 (10 mL × 2). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 100% EtOAc as the eluent.

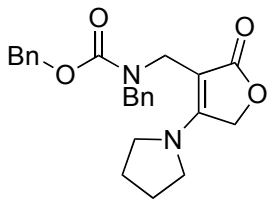


***tert*-Butyl ((2-Oxo-4-(pyrrolidin-1-yl)-2,5-dihydrofuran-3-yl)methyl)carbamate (47a).** After chromatographic purification, a white gel was obtained (74%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.21 (s, 1H), 4.59 (s, 2H), 4.07 (d, *J* = 5.8 Hz, 2H), 3.90 - 3.10 (br s, 4H), 2.00 (s, 4H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 176.8, 160.2, 155.6, 90.0, 79.2, 66.6, 60.4, 48.7, 33.9, 38.4; ESI-HRMS:

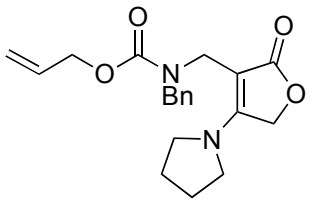
calc'd *m/e* for [M+H$^+$] $C_{14}H_{23}N_2O_4$: 283.1658, found 283.1648.



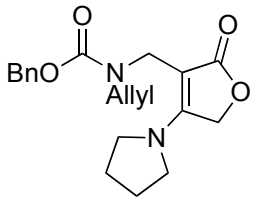
Phenyl ((2-Oxo-4-(pyrrolidin-1-yl)-2,5-dihydrofuran-3-yl)methyl)carbamate (47b). After chromatographic purification, a white gel was obtained (99%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.34 (t, *J* = 7.7 Hz, 2H), 7.18 (t, *J* = 7.3 Hz, 1H), 7.11 (d, *J* = 7.7 Hz, 2H), 5.86 (s, 1H), 4.62 (s, 2H), 4.20 (d, *J* = 6.0 Hz, 2H), 4.05 - 3.10 (br s, 4H), 1.98 (s, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 176.8, 160.1, 154.6, 151.0, 129.3, 125.3, 121.7, 89.6, 66.8, 60.4, 48.8, 34.8; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{16}H_{18}N_2NaO_4$: 325.1164, found 325.1170.



Benzyl Benzyl((2-oxo-4-(pyrrolidin-1-yl)-2,5-dihydrofuran-3-yl)methyl)carbamate (47c). After chromatographic purification, a yellow gel was obtained (86%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.26 - 7.08 (m, 10H), 5.13 (s, 2H), 4.48 (s, 2H), 4.29 (s, 2H), 4.08 (s, 2H), 3.60 - 2.80 (br s, 4H), 1.86 -1.58 (br s, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 176.9, 162.6, 156.0, 140.0, 139.6, 136.7, 128.4, 128.0, 127.5, 127.1, 126.5, 87.7, 67.2, 66.1, 64.4, 49.5, 48.4, 40.2; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{24}H_{27}N_2O_4$: 407.1971, found 407.1954.

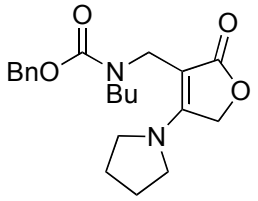


Allyl Benzyl((2-oxo-4-(pyrrolidin-1-yl)-2,5-dihydrofuran-3-yl)methyl)carbamate (47d). After chromatographic purification, a white gel was obtained (99%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.25 - 7.19 (m, 5H), 5.95 (s, 1H), 5.30 (m, 1H), 5.21 (m, 1H), 4.66 (s, 2H), 4.56 (s, 2H), 4.35 (s, 2H), 4.19 (m, 2H), 3.60 - 3.20 (br s, 4H), 1.89 (s, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 176.9, 162.6, 156.0, 139.6, 132.9, 128.0, 127.2, 126.6, 117.5, 87.8, 66.1, 49.6, 48.4, 40.1, 29.7, 25.0; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{20}H_{25}N_2O_4$: 357.1814, found 357.1802.

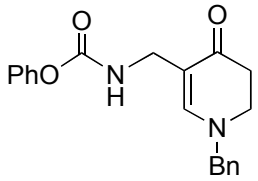


Benzyl Allyl((2-oxo-4-(pyrrolidin-1-yl)-2,5-dihydrofuran-3-yl)methyl)carbamate (47e). After chromatographic purification, a white gel was obtained (74%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.36 - 7.34 (m, 5H), 5.81 (s, 1H), 5.13 (s, 2H), 5.11 (m, 2H), 4.56 (s, 2H), 4.29 (s, 2H), 3.97 (d, *J* = 5.5 Hz, 2H), 3.96 - 3.02(br s, 4H), 1.94 (s, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 162.3, 156.0, 136.8, 134.0, 128.4, 127.9, 127.7, 116.2, 88.2, 67.0, 66.4, 48.7, 48.3, 39.4, 29.7, 25.1; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{20}H_{25}N_2O_4$: 357.1814,

found 357.1802.

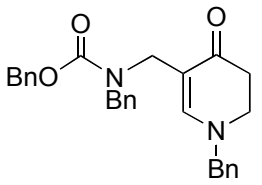


Benzyl Butyl((2-oxo-4-(pyrrolidin-1-yl)-2,5-dihydrofuran-3-yl)methyl)carbamate (47f). After chromatographic purification, a white gel was obtained (51%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.35 - 7.27 (m, 5H), 5.13 (s, 2H), 4.57 (s, 2H), 4.29 (s, 2H), 3.80 -2.98 (br s, 4H), 3.29 (t, *J* = 7.6 Hz, 2H), 1.93 (br, 4H), 1.54 (t, *J* = 7.1 Hz, 2H), 1.26 (m, 2H), 0.88 (m, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 176.9, 162.3, 156.1, 137.0, 128.4, 127.8, 127.7, 87.9, 66.3, 48.7, 45.2, 39.0, 30.3, 29.7, 25.1, 20.1, 13.9; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{21}H_{29}N_2O_4$: 373.2127, found 373.2120.

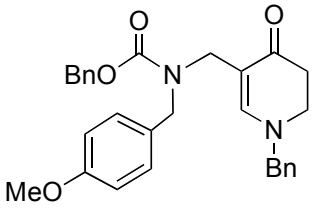


Phenyl ((1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)methyl)carbamate (47g). After chromatographic purification, a yellow oil was obtained (54%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.43 (s, 1H), 7.39 - 7.33 (m, 5H), 7.24 (m, 2H), 7.17 (t, *J* = 6.4 Hz, 1H), 7.12 (d, *J* = 7.4 Hz, 2H), 5.73 (s, 1H), 4.30 (s, 2H), 3.82 (d, *J* = 6.0 Hz, 2H), 3.27 (t, *J* = 9.6 Hz, 2H), 2.40 (t, *J* = 7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 190.7, 155.0, 151.2, 135.5, 129.2, 129.0, 128.4,

127.7, 125.1, 121.9, 121.7, 106.9, 60.0, 46.5, 39.3, 35.3, 29.7; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{20}H_{20}N_2NaO_3$: 359.1372, found 359.1380.



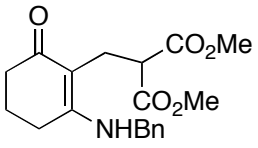
Benzyl Benzyl((1-benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)methyl)carbamate (47h). After chromatographic purification, a yellow oil was obtained (68%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.48 (s, 1H), 7.32 - 7.21 (m, 12H), 7.17 (m, 2H), 7.00 (m, 1H), 5.10 (s, 2H), 4.55 (s, 2H), 4.26 (s, 1H), 3.90 (s, 1H), 3.87 (d, *J* = 5.0 Hz, 2H), 3.16 (m, 2H), 2.30 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 190.0, 156.1, 138.8, 138.6, 136.9, 135.8, 135.7, 135.6, 129.0, 128.6, 128.4, 128.3, 128.2, 128.1, 127.7, 127.6, 127.5, 127.1, 126.9, 106.5, 67.1, 60.0, 50.6, 46.3, 43.7, 35.3; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{28}H_{28}N_2NaO_3$: 463.1998, found 463.2027.



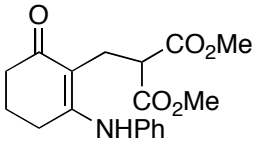
Benzyl ((1-Benzyl-4-oxo-1,4,5,6-tetrahydropyridin-3-yl)methyl)(4-methoxybenzyl)carbamate (47i). After chromatographic purification, a yellow oil was obtained (51%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.48 (s, 1H), 7.29 - 7.13 (m, 11H), 7.01 (m, 1H), 6.74 (m, 2H), 5.10 (s, 2H), 4.46 (s, 2H), 4.26 (s, 1H),

3.89 (s, 2H), 3.84 (s, 1H), 3.71 (s, 3H), 3.17 (m, 2H), 2.32 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 190.0, 158.7, 156.6, 156.1, 155.2, 136.9, 135.8, 130.8, 129.8, 129.2, 129.0, 128.6, 128.4, 128.38, 128.3, 128.2, 127.9, 127.8, 127.7, 127.4, 114.0, 113.7, 106.7, 106.5, 67.1, 60.0, 55.3, 50.0, 46.3, 43.2, 42.2, 35.4, 29.7; ESI-HRMS: calc'd *m/e* for $[M+Na^+]$ $C_{29}H_{30}N_2NaO_4$: 493.2103, found 493.2121.

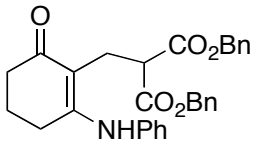
General Method for the Reaction of Enaminones with Malonates and Formaldehyde to form Methylmalonates 48 (Table 8). A suspension of enaminone, $LiClO_4$ (1.0 equiv), malonate diester (1.5 equiv) and acetic anhydride (1.0 equiv) in acetonitrile (10 mL) was stirred and pre-heated in a sealed screw-cap vial until the reaction mixture turned into a clear solution. To the reaction medium was then added paraformaldehyde (2.0 equiv), sealed and stirred at 60 °C. The reaction was monitored by TLC. The reaction was completed within 0.5 to 3 h. The reaction medium was then allowed to cool and washed with H_2O (20 mL). The aqueous phase was extracted with EtOAc (3 x 10 mL). The organic layers were combined, washed with brine and $MgSO_4$. The organic solvent was then removed under reduced pressure and the residue was purified by flash column flash column chromatography on silica gel, using 50% acetone/hexanes as the eluent.



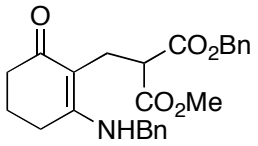
Dimethyl 2-((2-(Benzylamino)-6-oxocyclohex-1-enyl)methyl)malonate (48a). After chromatographic purification, a viscous colorless oil was obtained (93%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.38 - 7.34 (m, 2H), 7.30 - 7.26 (m, 3H), 6.35 (m, 1H), 4.43 (d, *J* = 6.1 Hz, 2H), 3.81(t, *J* = 7.2 Hz, 1H), 3.69 (s, 6H), 2.83 (d, 7.2 Hz, 2H), 2.39 (t, *J* = 6.2 Hz, 2H), 2.26 (t, *J* = 6.2 Hz, 2H), 1.84 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 194.9, 171.1, 162.7, 138.4, 129.0, 127.7, 126.9, 105.8, 52.7, 50.6, 47.2, 36.3, 25.8, 23.5, 21.2; ESI-HRMS: calc'd *m/e* for [M+Na$^+$] $C_{19}H_{23}NNaO_5$: 368.1474, found 368.1484; IR (neat, NaCl, cm^{-1}): 2951, 1733, 1575, 1436, 1409, 1357, 1299, 1246, 1202, 1158, 1118, 1031.



Dimethyl 2-((6-Oxo-2-(phenylamino)cyclohex-1-enyl)methyl)malonate (48b). After chromatographic purification, a viscous colorless oil was obtained (90%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.04 (s, 1H), 7.34 (m, 2H), 7.16 (m, 1H), 7.08 (m, 2H), 3.83 (t, *J* = 7.2 Hz, 1H), 3.76 (s, 6H), 2.92 (d, *J* = 7.2 Hz, 2H), 2.50 (t, *J* = 6.0 Hz, 2H), 2.37 (t, *J* = 6.0 Hz, 2H), 1.87 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.9, 171.3, 160.6, 139.3, 129.3, 125.0, 124.1, 109.0, 52.9, 50.9, 36.9, 27.4, 23.8, 22.2; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{18}H_{22}NO_5$: 332.1498, found 332.1505; IR (neat, NaCl, cm^{-1}): 2593, 1733, 1567, 1499, 1435, 1398, 1306, 1245, 1204, 1080, 1038.

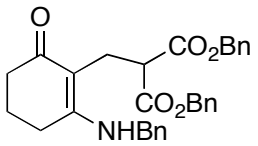


Dibenzyl 2-((6-Oxo-2-(phenylamino)cyclohex-1-enyl)methyl)malonate (48c). After chromatographic purification, a viscous light yellow oil was obtained (97%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.89 (s, 1H), 7.35-7.29 (m, 12H), 7.15 (m, 1H), 7.01 (m, 2H), 5.14 (q-like, *J* = 12.4 Hz, 4H), 3.95 (t, 1H, *J* = 7.4 Hz), 2.95 (d, 2H *J* = 7.4 Hz), 2.39 (t, J= 6.0 Hz, 2H), 2.32 (t, *J* = 6.0 Hz, 2H), 1.76 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 196.0, 170.5, 160.8, 139.1, 135.6, 129.3, 128.6, 128.4, 128.1, 125.1, 124.2,108.6, 67.4, 51.1, 36.8, 27.3, 23.7, 22.0; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{30}H_{30}NO_5$: 484.2124, found 484.2118; IR (neat, NaCl, cm^{-1}): 2948, 1736, 1578, 1498, 1454, 1431, 1396, 1304, 1233, 1199, 1153, 1080, 1030, 1003.

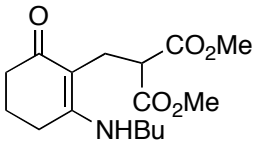


1-Benzyl 3-Methyl 2-((2-(Benzylamino)-6-oxocyclohex-1-enyl)methyl)malonate (48d). After chromatographic purification, a viscous light yellow oil was obtained (73%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.36 - 7.24 (m, 10H), 6.24 (t, *J* = 6.2 Hz, 1H), 5.15 (q-like, J= 12.4 Hz, 2H), 4.36 (d, *J* = 6.1 Hz, 2H), 3.89 (t, *J* = 7.6 Hz, 1H), 3.67 (s, 3H), 2.84 (m, 2H), 2.33 (m, 2H), 2.24 (m, 2H), 1.81 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 194.9, 170.7, 162.7, 138.4, 129.0, 128.6, 128.4, 128.1, 127.8, 127.0, 105.7, 67.2, 54.2, 52.7, 50.7,

47.2, 36.3, 25.8, 23.6, 21.2; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{25}H_{28}NO_5$: 422.1967, found 422.1973; IR (neat, NaCl, cm^{-1}): 2949, 1732, 1575, 1497, 1455, 1436, 1409, 1378, 1356, 1297, 1242, 1202, 1154, 1119, 1079, 1029.

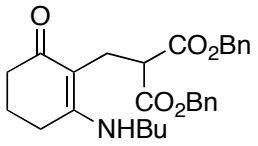


Dibenzyl 2-((2-(Benzylamino)-6-oxocyclohex-1-enyl)methyl)malonate (48e). After chromatographic purification, a viscous light yellow oil was obtained (90%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.36 - 7.22 (m, 15H), 6.20 (t, *J* = 6.1 Hz, 1H), 5.12 (q-like, *J* = 12.4 Hz, 4H), 4.30 (d, *J* = 6.1 Hz, 2H), 3.95 (t, *J* = 7.3 Hz, 1H), 2.87 (d, *J* = 7.3 Hz, 2H), 2.28 (t, *J* = 6.2 Hz, 2H), 2.22 (t, *J* = 6.3 Hz, 2H), 1.77 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): 194.9, 170.4, 162.7, 138.3, 135.7, 129.0, 128.6, 128.3, 128.1, 127.7, 127.0, 105.6, 67.2, 50.7, 47.1, 36.3, 25.7, 23.5, 21.1; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{31}H_{32}NO_5$: 498.2280, found 498.2275; IR (neat, NaCl, cm^{-1}): 2945, 1731, 1577, 1497, 1456, 1434, 1372, 1356, 1295, 1200, 1149, 1028.

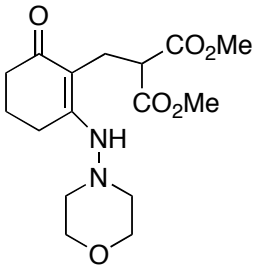


Dimethyl 2-((2-(Butylamino)-6-oxocyclohex-1-enyl)methyl)malonate (48f). After chromatographic purification, a viscous colorless oil (66%) was obtained. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.88 (s, 1H), 3.75 (t, *J* = 6.2 Hz, 1H), 3.69 (s,

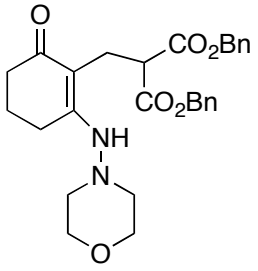
6H), 3.18 (m, 2H), 2.76 (d, *J* = 7.1 Hz, 2H), 2.41 (t, *J* = 6.2 Hz, 2H), 2.26 (t, *J* = 6.3 Hz, 2H), 1.87 (m, 2H), 1.57 (m, 2H), 1.40 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 194.4, 171.2, 163.0, 104.9, 52.8, 52.7, 50.6, 43.2, 36.3, 32.4, 25.8, 23.4, 21.3, 20.1, 13.9; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{16}H_{26}NO_5$: 312.1811, found 312.1801; IR (neat, NaCl, cm^{-1}): 2954, 1733, 1570, 1436, 1417, 1368, 1301, 1243, 1203, 1155, 1100.



Dibenzyl 2-((2-(Butylamino)-6-oxocyclohex-1-enyl)methyl)malonate (48g). After chromatographic purification, a viscous colorless oil was obtained (83%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.32 - 7.26 (m, 10H), 5.69 (t, *J* = 4.8 Hz, 1H), 5.13 (q-like, *J* = 12.4 Hz, 4H), 3.92 (t, *J* = 7.4 Hz, 1H), 3.04 (m, 2H), 2.81 (d, *J* = 7.3 Hz, 2H), 2.28 (t, *J* = 6.2 Hz, 2H), 2.22 (t, *J* = 6.1 Hz, 2H), 1.81 (m, 2H), 1.50 (m, 2H), 1.37 (m, 2H), 0.94 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 194.4, 170.6, 162.8, 135.8, 128.6, 128.3, 128.1, 104.7, 67.1, 50.7, 43.1, 36.3, 32.3, 25.8, 23.4, 21.2, 20.1, 13.79; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{28}H_{34}NO_5$: 464.2437, found 464.2444; IR (neat, NaCl, cm^{-1}): 2955, 1731, 1575, 1438, 1413, 1377, 1198, 1152.

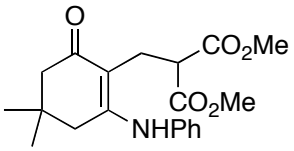


Dimethyl 2-((2-(Morpholinoamino)-6-oxocyclohex-1-enyl)methyl)malonate (48h). After chromatographic purification, a viscous light yellow oil was obtained (72%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.78 (s, 1H), 3.77 (t, *J* = 7.2 Hz, 1H), 3.76 (s, 4H), 3.71 (s, 6H), 2.76 (s, 4H), 2.74 (d, *J* = 7.2 Hz, 2H), 2.64 (t, *J* = 6.2 Hz, 2H), 2.27 (t, *J* = 6.2 Hz, 2H), 1.86 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.6, 171.1, 162.6, 103.6, 66.7, 56.9, 52.7, 50.6, 36.7, 25.2, 23.2, 21.4; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{16}H_{25}N_2O_6$: 341.1713, found 341.1699; IR (neat, NaCl, cm^{-1}): 2953, 2857, 1733, 1583, 1438, 1403, 1303, 1267, 1243, 1201, 1157, 1111, 1074, 1042, 1028.

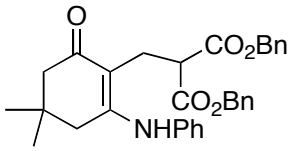


Dibenzyl 2-((2-(Morpholinoamino)-6-oxocyclohex-1-enyl)methyl)malonate (48i). After chromatographic purification, a viscous light yellow oil was obtained (86%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.33 - 7.25 (m, 10H), 6.64 (s, 1H), 5.14 (q-like, *J* = 12.5 Hz, 4H), 3.94 (t, *J* = 7.4 Hz, 1H), 3.90 - 3.40 (br s, 4H), 2.78 (d, *J* = 7.3 Hz, 2H), 2.58 (s, 4H), 2.54 (t, *J* = 6.2 Hz, 2H), 2.25 (t, *J* = 6.2 Hz, 2H),

1.82 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.5, 170.4, 162.6, 135.8, 128.6, 128.3, 127.9, 103.5, 67.2, 66.6, 56.7, 50.8, 36.7, 25.2, 23.1, 21.3; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{28}H_{33}N_2O_6$: 493.2339, found 493.2342; IR (neat, NaCl, cm^{-1}): 2917, 2850, 1732, 1584, 1497, 1455, 1402, 1385, 1266, 1233, 1200, 1152, 1112, 1076.

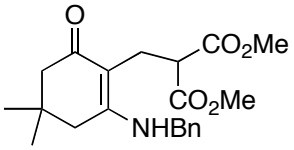


Dimethyl 2-((4,4-Dimethyl-6-oxo-2-(phenylamino)cyclohex-1-enyl)methyl)-malonate (48j). After chromatographic purification, a viscous colorless oil was obtained (83%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.94 (s, 1H), 7.35 (m, 2H), 7.16 (m, 1H), 7.06 (m, 2H), 3.88 (t, *J* = 7.4 Hz, 1H), 3.75 (s, 6H), 2.93 (d, *J* = 7.4 Hz, 2H), 2.36 (s, 2H), 2.23 (s, 2H), 0.97 (s, 6H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.5, 171.3, 158.4, 139.3, 129.4, 124.9, 123.9, 108.0, 52.9, 50.7, 50.5, 40.8, 32.8, 28.1, 23.8; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{20}H_{26}NO_5$: 314.1392, found 314.1385; IR (neat, NaCl, cm^{-1}): 2954, 1735, 1579, 1498, 1436, 1395, 1321, 1270, 1243, 1150.

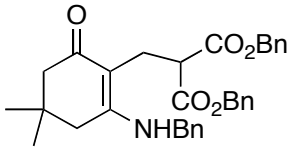


Dibenzyl 2-((4,4-Dimethyl-6-oxo-2-(phenylamino)cyclohex-1-enyl)methyl)-malonate (48k). After chromatographic purification, a viscous light yellow oil

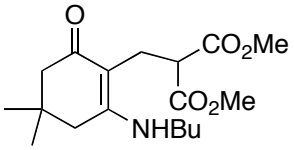
was obtained (90%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.90 (s, 1H), 7.34 (m, 2H), 7.29 (m, 10H), 7.16 (m, 1H), 7.00 (m, 2H), 5.16 (dt, *J* = 3.2, 14.0 Hz, 4H), 3.99 (t, *J* = 7.5 Hz, 1H), 2.95 (d, *J* = 7.5 Hz, 2H), 2.27 (s, 2H), 2.18 (s, 2H), 0.91 (s, 6H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.5, 170.5, 158.4, 139.3, 135.5, 129.3, 128.7, 128.4, 128.2, 124.9, 124.0, 107.9, 67.4, 51.0, 50.5, 40.7, 32.7, 28.1, 23.7; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{32}H_{34}NO_5$: 512.2437, found 512.2431; IR (neat, NaCl, cm^{-1}): 2956, 1733, 1579, 1498, 1455, 1395, 1384, 1321, 1271, 1227, 1147, 1071.



Dimethyl 2-((2-(Benzylamino)-4,4-dimethyl-6-oxocyclohex-1-enyl)methyl)-malonate (48l). After chromatographic purification, a viscous colorless oil was obtained (95%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 (m, 2H), 7.9 (m, 3H), 6.20 (m, 1H), 4.43 (d, *J* = 6.2 Hz, 2H), 3.82 (t, *J* = 7.4 Hz, 1H), 3.69 (s, 6H), 2.84 (d, J= 7.3 Hz, 2H), 2.24 (s, 2H), 2.16 (s, 2H), 0.96 (s, 6H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 194.4, 171.1, 161.0, 138.5, 129.0, 127.8, 126.9, 104.5, 52.7, 50.5, 50.0, 47.1, 39.3, 31.8, 28.5, 23.4; ESI-HRMS: ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{21}H_{28}NO_5$: 374.1967, found 374.1964; IR (neat, NaCl, cm^{-1}): 2953, 1732, 1576, 1436, 1407, 1357, 1325, 1271, 1245, 1150, 1118, 1036.

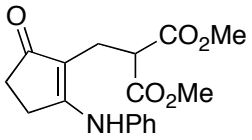


Dibenzyl 2-((2-(Benzylamino)-4,4-dimethyl-6-oxocyclohex-1-enyl)methyl)malonate (48m). After chromatographic purification, a viscous light yellow oil was obtained (88%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 - 7.23 (m, 15H), 6.10 (t, *J* = 6.5 Hz, 1H), 5.11 (q-like, *J* = 12.4 Hz, 4H), 4.32 (d, *J* = 6.3 Hz, 2H), 3.95 (t, *J* = 7.4 Hz, 1H), 2.87 (d, *J* = 7.4 Hz, 2H), 2.13 (s, 2H), 2.10 (s, 2H), 0.92 (s, 6H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 194.4, 170.4, 160.9, 138.5, 135.6, 129.0, 128.6, 128.3, 128.2, 127.7, 126.9, 104.3, 67.2, 50.8, 49.9, 47.0, 39.2, 31.8, 28.5, 23.3; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{33}H_{36}NO_5$: 526.2593, found 526.2586; IR (neat, NaCl, cm^{-1}): 2955, 1731, 1577, 1497, 1455, 1407, 1356, 1324, 1271, 1233, 1147, 1028.

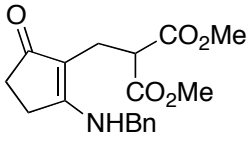


Dimethyl 2-((2-(Butylamino)-4,4-dimethyl-6-oxocyclohex-1-enyl)methyl)malonate (48n). After chromatographic purification, a viscous colorless oil was obtained (88%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.73 (s, 1H), 3.77 (t, *J* = 7.3 Hz, 1H), 3.69 (s, 6H), 3.18 (m, 2H), 2.77 (d, *J* = 7.3 Hz, 2H), 2.25 (s, 2H), 2.16 (s, 2H), 1.57 (m, 2H), 1.40 (m, 2H), 1.02 (s, 6H), 0.96 (t, 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 193.8, 171.2, 161.1, 103.5, 52.6, 50.5, 49.9, 43.2, 39.5, 32.7, 31.8, 28.6, 23.3, 20.1, 13.9; ESI-HRMS: calc'd *m/e*

for [M+H$^+$] $C_{18}H_{30}NO_5$: 374.1967, found 374.1964; IR (neat, NaCl, cm^{-1}): 2953, 1733, 1576, 1436, 1407, 1357, 1325, 1271, 1245, 1150, 1118, 1036.



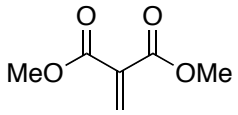
Dimethyl 2-((5-Oxo-2-(phenylamino)cyclopent-1-enyl)methyl)malonate (48o). After chromatographic purification, a viscous colorless oil was obtained (96%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.20 (s, 1H), 7.34 (t, *J* = 7.7 Hz, 2H), 7.14 (m, 3H), 3.75 (s, 6H), 3.72 (t, *J* = 7.2 Hz, 1H), 2.78 (d, J= 7.0 Hz, 2H), 2.70 (s, 2H), 2.37 (s, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 202.9, 171.5, 170.8, 139.3, 129.5, 124.9, 122.3, 111.6, 52.9, 50.3, 33.2, 26.3, 21.7; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{17}H_{20}NO_5$: 318.1341, found 318.1344; IR (neat, NaCl, cm^{-1}): 2953, 1734, 1667, 1581, 1499, 1435, 1410, 1340, 1293, 1240, 1158, 1079, 1063.



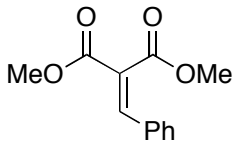
Dimethyl 2-((2-(Benzylamino)-5-oxocyclopent-1-enyl)methyl)malonate (48p). After chromatographic purification, a viscous colorless oil was obtained (94%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.36 (m, 2H), 7.29 (m, 3H), 6.43 (s, 1H), 4.47 (d, *J* = 6.4 Hz, 2H), 3.71 (t, *J* = 6.9 Hz, 1H), 2.69 (d, J= 7.2 Hz, 2H), 2.54 (s, 2H), 2.33 (s, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 202.3, 174.1, 170.6, 138.0, 129.0, 127.9, 127.1, 127.0, 108.6, 52.8, 50.3, 32.9, 24.9, 21.8; ESI-HRMS: calc'd

m/e for [M+H$^+$] $C_{18}H_{22}NO_5$: 332.1498, found 332.1492; IR (neat, NaCl, cm^{-1}): 2952, 1733, 1669, 1594, 1578, 1496, 1436, 1353, 1279, 1238, 1156, 1118, 1077, 1056, 1030.

Mechanistic Studies for the $LiClO_4$-mediated Formation of Enaminone Methylmalonate (**Figure 56**)



Dimethyl 2-Methylenemalonate. Dimethyl malonate (11.5 mL, 100.0 mmol) was dissolved in acetic acid (45 mL). The reaction was then heated at 100 °C for 3-4 h. After cooling, the salts were filtered off and acetic acid was removed, using bulb-to-bulb distillation (bp 50-60 °C, 100 mmHg). Further distillation (bp 120-130 °C, 20 mmHg missing) gave a colorless oil (3.7 g, 25%). The product was taken into the reaction with the enaminone immediately after it was isolated, in order to avoid polymerization. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.56 (s, 2H), 3.83 (s, 6H); Polymer: δ 3.75 (s, 3H), 3.40 (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 167.1, 135.2, 52.7; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_6H_9O_4$: 145.0501, found 145.0500.



Dimethyl 2-Benzylidenemalonate. To a solution of benzaldehyde (6.6 mL, 60 mmol) in DMSO (20 mL), proline (690.0 mg, 6.0 mmol) was added. The mixture

was stirred for 5 min before the addition of dimethyl malonate (13.7 mL, 120 mmol). After stirring at room temperature for 24 h, the reaction was diluted with EtOAc (60 mL) and washed with water (60 mL × 2). The combined organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography on silica gel, using 50% acetone:hexanes (v/v) as the eluent. After purification, a viscous oil was obtained (12.8 g, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.78 (s, 1H), 7.42 - 7.40 (m, 5H), 3.86 (s, 3H), 3.85 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 167.3, 164.6, 143.1, 132.9, 130.8, 129.5, 129.0, 125.6, 52.8; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{12}H_{13}O_4$: 221.0814, found 221.0805.

Four reactions were set up parallely, utilizing the enaminone (3-(benzylamino)cyclohex-2-enone) to react with dimethyl 2-methylenemalonate and dimethyl 2-benzylidenemalonate synthesized above, respectively:

a) A solution of enaminone (50.2 mg, 0.25 mM), $LiClO_4$ (30 mg, 0.25 mM), and dimethyl 2-methylenemalonate (54 mg, 0.38 mM) was heated in a sealed screw-cap vial at 60 °C.

b) A solution of enaminone (50.2 mg, 0.25 mM) and dimethyl 2-methylenemalonate (54 mg, 0.38 mM) was heated in a sealed screw-cap vial at 60 °C.

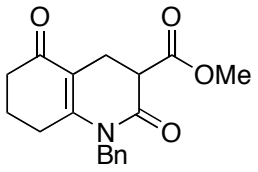
c) A solution of enaminone (50.2 mg, 0.25 mM), $LiClO_4$ (30 mg, 0.25 mM), and dimethyl 2-benzylidenemalonate (83 mg, 0.38 mM) was heated in a sealed screw-cap vial at 60 °C.

d) A solution of enaminone (50.2 mg, 0.25 mM) and dimethyl 2-benzylidenemalonate (83 mg, 0.38 mM) was heated in a sealed screw-cap vial at 60 °C.

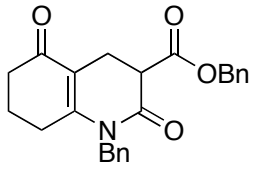
The reactions were monitored by TLC for product formation. After starting material was completely consumed, the reaction medium was then allowed to cool and washed with H_2O (20 mL). The aqueous phase was extracted with EtOAc (3 x 10 mL). The organic layers were combined, washed with brine and $MgSO_4$. The organic solvent was then removed under reduced pressure and the residue was purified by flash column flash column chromatography on silica gel. Only reactions a and b yielded desired adducts in a yield of 90%, as the result of this study.

General Method for the Synthesis of *N*-Substituted 2,5-Dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylates 49 (Table 9). The methylmalonate **48** was dissolved (10 mL) in a screw-cap vial. Dichloroethane was selected as the solvent for those compounds derived from dimethyl malonate, while toluene was used for those derived from dibenzyl malonate. To the solution was then slowly added trifluoroacetic acid (30.0 equiv). The vial was sealed and stirred at 90 °C. The reaction was monitored by TLC. The reaction time varied from 0.5 to 3 hr. The reaction medium was then allowed to cool and washed with saturated $NaHCO_3$ (20 mL). The aqueous phase was extracted with CH_2Cl_2 or EtOAc (3 x 10 mL). The organic layers were combined and washed with brine and dried over $MgSO_4$. The organic solvent was then removed under reduced pressure

and the residue was purified by flash column flash column chromatography on silica gel, using 20% EtOAc:hexanes (v/v) as the eluent.

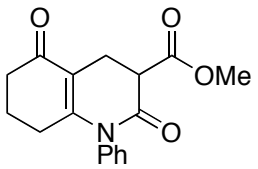


Methyl 1-Benzyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (49a). After chromatographic purification, a light yellow oil was obtained (79%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.27 (m, 2H), 7.20 (m, 1H), 7.11 (m, 2H), 4.96 (q-like, *J* = 16.4 Hz, 2H), 3.70 (s, 3H), 3.57 (t, *J* = 8.3 Hz, 1H), 3.00 (dd, *J* = 8.4, 16.9 Hz, 1H), 2.78 (dd, *J* = 8.5, 16.9 Hz, 1H), 2.42 (t, *J* = 6.3 Hz, 2H), 2.28 (t, *J* = 6.2 Hz, 2H), 1.88 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.8, 169.6, 167.2, 154.7, 136.7, 128.98, 127.7, 126.2, 115.0, 52.8, 47.4, 45.7, 36.0, 26.5, 21.8, 20.8; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{18}H_{20}NO_4$: 360.1811, found 360.1801; IR (neat, NaCl, cm^{-1}): 2953, 1743, 1692, 1654, 1622, 1497, 1454, 1437, 1392, 1365, 1326, 1299, 1280, 1259, 1195, 1167, 1128.

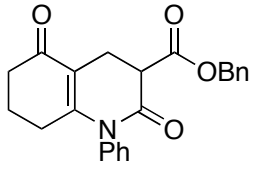


Benzyl 1-Benzyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (49b). After chromatographic purification, a light yellow oil was obtained (70%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 -7.24 (m, 8H), 7.16 (m, 2H), 5.20 (q-like, *J* = 12.2 Hz, 2H), 5.00 (q-like, *J* = 16.5 Hz, 2H), 3.68 (t, *J* = 7.0 Hz, 1H), 3.15 (dd,

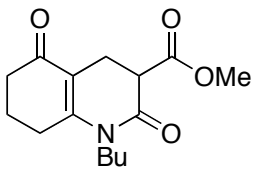
J = 7.5, 16.8 Hz, 1H), 2.80 (dd, *J* = 6.6, 16.8 Hz, 1H), 2.41 (m, 2H), 2.26 (m, 2H), 1.84 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.6, 168.9, 167.1, 154.7, 136.6, 135.4, 129.0, 128.6, 128.4, 128.3, 127.5, 126.1, 115.0, 67.3, 47.5, 45. 6, 35.9, 26.3, 21.6, 20.8; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{24}H_{24}NO_4$: 390.1705, found 390.1700; IR (neat, NaCl, cm^{-1}): 2953, 1738, 1691, 1652, 1622, 1497, 1455, 1428, 1390, 1364, 1297, 1258, 1194, 1164, 1126.



Methyl 2,5-Dioxo-1-phenyl-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (49c). After chromatographic purification, a white solid was obtained (84%, mp: 120 - 121 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.48 - 7.40 (m, 3H), 7.17 (s, 2H), 3.76 (s, 3H), 3.68 (t, *J* = 7.6 Hz, 1H), 3.18 (dd, *J* = 8.4, 17.0 Hz, 1H), 2.90 (dd, *J* = 8.6, 17.0 Hz, 1H), 2.39 (t, *J* = 6.0 Hz, 2H), 2.09 (t, *J* = 6.0 Hz, 2H), 1.93 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 196.0, 169.6, 167.1, 154.9, 137.0, 129.7, 129.0, 113.9, 52.8, 47.6, 36.3, 28.0, 21.9, 21.1; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{17}H_{18}NO_4$: 300.1236, found 300.1228; IR (neat, NaCl, cm^{-1}): 2953, 1742, 1702, 1652, 1625, 1594, 1493, 1380, 1271, 1244, 1154.

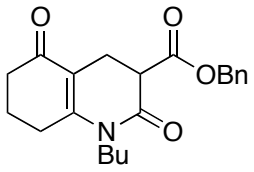


Benzyl 2,5-Dioxo-1-phenyl-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (49d). After chromatographic purification, a light yellow solid was obtained (49%, mp: 117 - 118 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.43 (m, 3H), 7.35 (m, 5H), 7.09 (m, 2H), 5.20 (q-like, *J* = 12.1 Hz, 2H), 3.73 (t, *J* = 6.5 Hz, 1H), 3.30 (dd, *J* = 6.5, 17.0 Hz, 1H), 2.85 (dd, *J* = 6.6, 16.9 Hz, 1H), 2.33 (m, 2H), 1.99 (m, 2H), 1.81 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.8, 168.8, 167.0, 154.8, 136.9, 135.3, 129.6, 128.9, 128.6, 128.5, 128.4, 113.8, 67.4, 47.6, 36.1, 27.9, 21.7, 21.2; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{23}H_{22}NO_4$: 376.1549, found 376.1546; IR (neat, NaCl, cm^{-1}): 2951, 1740, 1704, 1652, 1624, 1595, 1491, 1455, 1379, 1350, 1311, 1271, 1244, 1197, 1155.

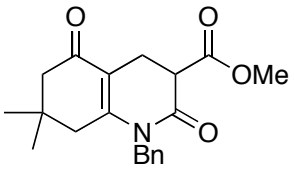


Methyl 1-Butyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (49e). After chromatographic purification, a viscous yellow oil was obtained (57%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.74 (s, 3H), 3.69 (m, 2H), 3.48 (dd, *J* = 6.8, 9.3 Hz, 1H), 2.94 (dd, *J* = 9.3, 17.0 Hz, 1H), 2. 79 (dd, *J* = 6.8, 16.6 Hz, 1H), 2.60 (m, 2H), 2.40 (t, *J* = 6.2 Hz, 2H), 2.07 (m, 2H), 1.54 (m, 2H), 1.35 (m, 2H), 0.94 (t, *J* = 7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.66, 169.62, 166.79, 154.34, 114.73, 52.60, 47.31, 42.60, 35.98, 31.24, 26.20, 21.84, 20.64, 20.08, 13.74; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{15}H_{22}NO_4$: 280.1549, found

280.1544; IR (neat, NaCl, cm^{-1}): 2957, 1744, 1690, 1652, 1618, 1435, 1393, 1279, 1245, 1201, 1121.

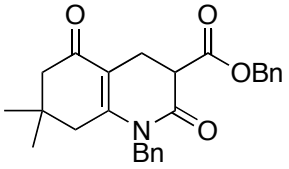


Benzyl 1-Butyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (1f). After chromatographic purification, a viscous colorless oil was obtained (60%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.36 - 7.29 (m, 5H), 5.16 (q-like, *J* = 12.2 Hz, 2H), 3.68 (m, 2H), 3.52 (dd, *J* = 6.6, 8.0 Hz, 1H), 3.04 (dd, *J* = 8.0, 16.7 Hz, 1H), 2.73 (dd, *J* = 6.4, 17.2 Hz, 1H), 2.51 (m, 2H), 2.34 (m, 2H), 1.96 (m, 2H), 1.50 (m, 2H), 1.34 (m, 2H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.55, 168.94, 166.72, 154.45, 135.43, 128.55, 128.37, 128.31, 114.66, 67.18, 47.46, 42.56, 35.93, 31.18, 26.12, 21.74, 20.76, 20.06, 13.74; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{21}H_{26}NO_4$: 356.1862, found 356.1861; IR (neat, NaCl, cm^{-1}): 2958, 1741, 1691, 1652, 1618, 1498, 1456, 1394, 1278, 1245, 1197, 1172, 1121.

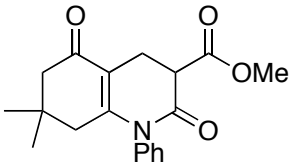


Methyl 1-Benzyl-7,7-dimethyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (49g). After chromatographic purification, a viscous colorless oil was obtained (67%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.34 (m, 2H), 7.26 (m,

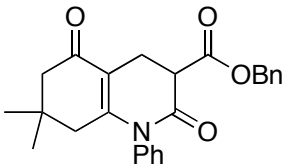
1H), 7.17 (m, 2H), 5.00 (q-like, *J* = 16.4 Hz, 2H), 3.75 (s, 3H), 3.64 (t, *J* = 6.9 Hz, 1H), 3.14 (dd, *J* = 7.2, 16.8 Hz, 1H), 2.80 (dd, *J* = 6.6, 16.8 Hz, 1H), 2.34 (s, 2H), 2.22 (s, 2H), 0.95 (s, 3H), 0.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.7, 169.6, 167.4, 152.9, 136.8, 129.1, 127.7, 126.1, 114.1, 52.8, 49.8, 47.5, 45.6, 40.1, 33.2, 28.9, 27.5, 20.6; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{20}H_{24}NO_4$: 342.1705, found 342.1698; IR (neat, NaCl, cm^{-1}): 2956, 1741,1693, 1653, 1625, 1497, 1454, 1439, 1386, 1303, 1280, 1252, 1204, 1168.



Benzyl 1-Benzyl-7,7-dimethyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (49h). After chromatographic purification, a viscous colorless oil was obtained (64%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.35 - 7.25 (m, 8H), 7.16 (m, 2H), 5.18 (q-like, J= 12.2 Hz, 2H), 5.00 (q-like, *J* = 16.6 Hz, 2H), 3.69 (t, *J* = 6.8 Hz, 1H), 3.19 (dd, *J* = 7.0, 16.8 Hz, 1H), 2.79 (dd, *J* = 4.7, 16.8 Hz, 1H), 2.27 (q-like, *J* = 10.2 Hz, 2H), 2.15 (q-like, *J* = 16.3 Hz, 2H), 0.90 (s, 3H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.6, 169.0, 167.3, 153.0, 136.7, 135.4, 129.0, 128.7, 128.5, 128.4, 127.6, 126.0, 114.0, 67.4, 49.7, 47.6, 45.5, 40.0, 33.0, 28.6, 27.7, 20.7; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{26}H_{28}NO_4$: 418.2018, found 418.2021; IR (neat, NaCl, cm^{-1}): 2959, 1739, 1694, 1653, 1625, 1497, 1455, 1386, 1369, 1303, 1252, 1213, 1169, 1124.

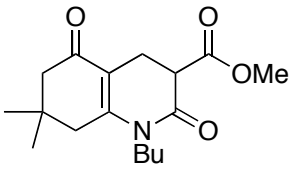


Methyl 7,7-Dimethyl-2,5-dioxo-1-phenyl-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (49i). After chromatographic purification, a white solid was obtained (64%, mp: 123 - 124 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.50 - 7.42 (m, 3H), 7.21 - 7.10 (m, 2H), 3.75 (s, 3H), 3.69 (t, *J* = 5.8 Hz, 1H), 3.25 (dd, *J* = 6.6, 16.9 Hz, 1H), 2.86 (dd, *J* = 6.6, 16.9 Hz, 1H), 2.26 (s, 2H), 1.95 (q-like, *J* = 17.6 Hz, 2H), 0.97 (s, 3H), 0.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.8, 169.5, 167.2, 153.0, 136.9, 129.7, 128.9, 128.5, 113.0, 52.7, 49.9, 47.5, 41.5, 33.2, 28.8, 27.4, 20.8; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{19}H_{22}NO_4$: 328.1549, found 328.1540; IR (neat, NaCl, cm^{-1}): 2956, 1740, 1704, 1654, 1628, 1596, 1492, 1437, 1380, 1317, 1292, 1263, 1239, 1144.

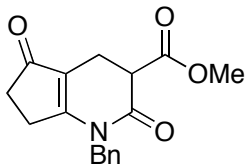


Benzyl 7,7-Dimethyl-2,5-dioxo-1-phenyl-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (49j). After chromatographic purification, a white solid was obtained (64%, mp: 126 - 127 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.43 (m, 3H), 7.34 (m, 5H), 7.07 (m, 2H), 5.18 (q-like, *J* = 12.2 Hz, 2H), 3.73 (t, *J* = 6.3 Hz, 1H), 3.33 (dd, *J* = 5.8, 16.9 Hz, 1H), 2.84 (dd, *J* = 6.3, 16.9 Hz, 1H), 2.19 (q-like, *J* = 16.4 Hz, 2H), 1.88 (q-like, *J* = 17.0 Hz, 2H), 0.94 (s, 3H), 0.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.7, 168.8, 167.2, 152.9, 136.9, 135.2, 129.6,

128.9, 128.7, 128.5, 128.5, 112.8, 67.5, 49.8, 47.6, 41.4, 33.0, 28.6, 27.6, 20.9; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{25}H_{26}NO_4$: 404,1862, found 404.1864; IR (neat, NaCl, cm^{-1}): 2958, 1740, 1705, 1654, 1628, 1596, 1492, 1455, 1379, 1351, 1317, 1292, 1240, 1262, 1173, 1143.

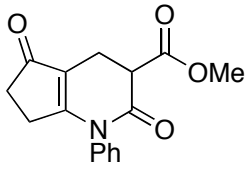


Methyl 1-Butyl-7,7-dimethyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate (49k). After chromatographic purification, a viscous light yellow oil was obtained (62%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.71 (s, 3H), 3.68 (m, 2H), 3.48 (dd, *J* = 6.6, 8.0 Hz, 1H), 3.00 (dd, *J* = 8.1, 16.7 Hz, 1H), 2.72 (dd, *J* = 6.6, 16.8 Hz, 1H), 2.42 (s, 2H), 2.27 (s, 2H), 1.53 (m, 2H), 1.35 (m, 2H), 1.10 (s, 3H), 1.07 (s, 3H), 0.94 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.6, 169.7, 167.1, 152.6, 113.8, 52.7, 49.8, 47.4, 42.5, 40.1, 33.2, 31.4, 29.0, 27.9, 20.5, 20.2, 13.9; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{17}H_{26}NO_4$: 308.1862, found 308.1868; IR (neat, NaCl, cm^{-1}): 2958, 2872, 1745, 1692, 1653, 1623, 1439, 1391, 1310, 1279, 1248, 1206, 1173, 1118, 1032, 1009.

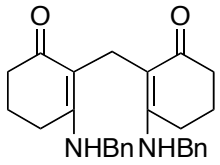


Methyl 1-Benzyl-2,5-dioxo-2,3,4,5,6,7-hexahydro-1H-cyclopenta[b]pyridine-3-carboxylate (49l). After chromatographic purification, a white solid was

obtained (62%, mp: 134 - 135 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 - 7.29 (m, 3H), 7.24 (m, 2H), 4.96 (q-like, *J* = 15.8 Hz, 2H), 3.77 (s, 3H), 3.72 (t, *J* = 7.6 Hz, 1H), 2.96 (dd, *J* = 7.3, 17.3 Hz, 1H), 2.75 (dd, *J* = 7.9, 17.0 Hz, 1H), 2.65 (m, 2H), 2.46 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 201.5, 169.6, 168.4, 167.4, 136.3, 129.1, 128.0, 126.9, 126.8, 116.9, 53.0, 47.5, 46.5, 34.3, 25.4, 19.8; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{17}H_{18}NO_4$: 300.1236, found 300.1230; IR (neat, NaCl, cm^{-1}): 2953, 2928, 1742, 1687, 1640, 1496, 1437, 1412, 1363, 1322, 1281, 1253, 1213, 1167, 1137, 1123, 1027, 1083.



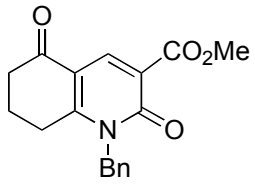
Methyl 2,5-Dioxo-1-phenyl-2,3,4,5,6,7-hexahydro-1H-cyclopenta[b]pyridine-3-carboxylate (49m). After chromatographic purification, a white solid was obtained (49%, mp: 100 - 101 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.50 - 7.44 (m, 3H), 7.24 (m, 2H), 3.79 (t, *J* = 6.4 Hz, 1H), 3.78 (s, 3H), 3.06 (dd, *J* = 6.3, 17.1 Hz, 1H), 2.83 (dd, *J* = 7.8, 17.1 Hz, 1H), 2.45 (m, 2H), 2.33 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 201.9, 169.7, 168.6, 167.3, 136.1, 129.8, 129.4, 128.3, 116.1, 53.1, 47.6, 34.3, 26.0, 20.1; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{16}H_{16}NO_4$: 286.1079, found 286.1074; IR (neat, NaCl, cm^{-1}): 2954, 2929, 1743, 1690, 1641, 1595, 1564, 1492, 1439, 1395, 1352, 1317, 1293, 1257, 1140, 1024, 1000.



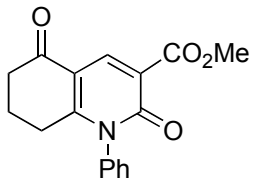
2,2'-Methylenebis(3-(benzylamino)cyclohex-2-enone). The enaminone was dissolved in acetonitrile, and paraformaldehyde and trimethylsilyl chloride (1.0 equiv) were added. The reaction was stirred 60 °C for 2 h. Upon disappearance of the starting material, the solvent was removed *in vacuo*, and the crude material was purified by flash column flash column chromatography on silica gel, using 50% acetone:hexanes (v/v) as the eluent. After purification, a light yellow oil was obtained (74%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 9.51 (s, 2H), 7.33 - 7.20 (m, 10H), 4.48 (d, J= 6.4 Hz, 4H), rotomer (-CH_2 bridge) [4.11, 3.77, 3.44], 2.36 (s, 4H), 2.27 (s 4H), 1.82 (m, 4H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 194.9, 166.9, 139.1, 128.8, 127.3, 126.7, 108.9, 63.8, 52.9, 46.8, 35.7, 25.6, 21.5, 17.7; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{27}H_{31}N_2O_2$: 415.2386, found 415.2390; IR (neat, NaCl, cm^{-1}): 3262, 3171, 3030, 2946, 1734, 1610, 1560, 1478, 1433, 1355, 1317, 1201, 1127, 1106, 1051, 1011.

General Procedure for the Oxidation of Methyl 2,5-Dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylates to Form 1-Substituted Methyl 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylates 50. The 1-substituted methyl 2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carboxylate **49** (1.4 mmol) was dissolved in MeOH (10 mL). To the stirring solution, triethylamine (1.1 mL, 4.2 equiv) was added. Next, the solution was purged with oxygen gas through a

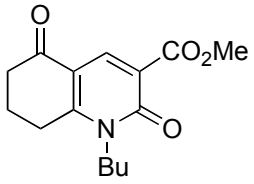
cannula. The mixture was then heated to 80 °C and allowed to react for 3 h. The reaction was then allowed to cool to room temperature, and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 75% EtOAc:hexanes (v/v) as the eluent.



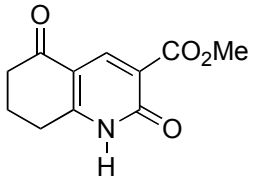
Methyl 1-Benzyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (50a). After chromatographic purification, a yellow amorphous solid was obtained (94%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.79 (s, 1H), 7.35 - 7.28 (m, 3H), 7.16 (d, *J* = 6.8 Hz, 2H), 5.42 (s, 2H), 3.90 (s, 3H), 2.91 (t, *J* = 6.2 Hz, 2H), 2.52 (t, *J* = 6.4 Hz, 2H), 2.08 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 193.0, 164.8, 160.8, 159.8, 141.6, 134.9, 129.1, 128.0, 126.5, 118.5, 114.0, 52.4, 47.6, 36.1, 27.9, 21.0; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{18}H_{18}NO_4$: 312.1236, found 312.1232; IR (neat, NaCl, cm^{-1}): 2953, 1739, 1706, 1665, 1598, 1540, 1496, 1438, 1388, 1357, 1338, 1318, 1249, 1233, 1192, 1147, 1116, 1047, 1017, 924, 916, 797, 734, 701.



Methyl 2,5-Dioxo-1-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (50b). After chromatographic purification, a white solid was obtained (24%, mp: 142 - 144 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.81 (s, 1H), 7.55 - 7.49 (m, 3H), 7.18 (m, 2H), 3.86 (s, 3H), 2.52 (t, *J* = 6.4 Hz, 2H), 2.48 (t, *J* = 6.2 Hz, 2H), 2.03 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 193.2, 164.8, 164.7, 160.9, 159.7, 142.0, 137.1, 130.1, 129.6, 129.1, 127.6, 119.2, 113.6, 52.3, 36.2, 29.5, 21.1; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{17}H_{16}NO_4$: 298.1079, found 298.1066.

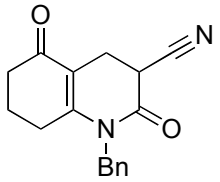


Methyl 2,5-Dioxo-1-butyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (50c). After chromatographic purification, a yellow oil was obtained (60%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 8.70 (s, 1H), 4.05 (t, *J* = 7.9 Hz, 2H), 3.86 (s, 3H), 3.00 (t, *J* = 6.2 Hz, 2H), 2.55 (t, *J* = 7.2 Hz, 2H), 2.20 (m, 2H), 1.67 (m, 2H), 1.42 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 193.1, 164.9, 159.9, 159.3, 141.2, 118.1, 113.8, 52.3, 44.9, 36.2, 30.3, 26.7, 21.1, 20.3, 13.6; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{15}H_{20}NO_4$: 278.1392, found 278.1385.

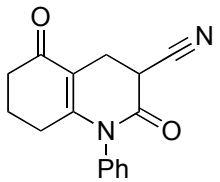


Methyl 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (50d). After chromatographic purification, a white solid was obtained (25%, mp: 226 - 228 °C). ^{1}H NMR (MeOD, 400 MHz, ppm): δ 8.68 (s, 1H), 3.87 (s, 3H), 2.94 (t, *J* = 6.2 Hz, 2H), 2.59 (t, *J* = 6.1 Hz, 2H), 2.18 (m, 2H); ^{13}C NMR (MeOD, 400 MHz, ppm): δ 195.7, 166.0, 163.1, 162.1, 143.9, 119.1, 114.4, 52.5, 37.8, 28.0, 22.0; ESI-HRMS: calc'd *m/e* for [M+H^{+}] $C_{11}H_{12}NO_4$: 222.0766, found 222.0762.

General Procedure for the Preparation of 3-Cyano-4,6,7,8-tetrahydroquinoline-2,5-diones 51 (Table 10). The enaminone was dissolved in acetonitrile (10 mL per 0.1 mmol), and stirred at room temperature. To the stirring solution, lithium perchlorate (1 equiv relative to the enaminone) was added, followed by the addition of PPh_3/PBu_3 (0.5 equiv), methyl cyanoacetate (1.5 equiv) and paraformaldehyde (1.0 equiv) in sequence. The reaction was heated to 60 °C in a sealed vessel and monitored by TLC. When the starting material was completely consumed, the reaction was quenched with saturated sodium bicarbonate. The organic layer was separated and aqueous phase was extracted with CH_2Cl_2 (10 mL × 2). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel, using 25% EtOAc:hexanes (v/v) as the eluent.

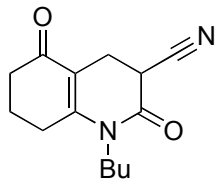


1-Benzyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitrile (51a). After chromatographic purification, a colorless oil was obtained (85%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 - 7.27 (m, 3H), 7.13 (m, 2H), 5.12 (d, *J* = 16.3 Hz, 1H), 4.91 (d, 16.3 Hz, 1H), 3.75 (dd, *J* = 6.3, 11.3 Hz, 1H), 3.17 (ddd, *J* = 1.4, 4.8, 15.3 Hz, 1H), 2.87 (m, 1H), 2.53 (m, 2H), 2.38 (t, *J* = 6.2 Hz, 2H), 1.99 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.1, 163.0, 154.6, 135.8, 129.2, 127.9, 126.1, 115.6, 114.6, 46.0, 35.7, 34.4, 26.3, 21.8, 21.5; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{17}H_{17}N_2O_2$: 281.1290, found 281.1281; IR (neat, NaCl, cm^{-1}): 2959, 2890, 2256, 1756, 1700, 1655, 1627, 1497, 1454, 1387, 1307, 1255, 1249, 1207, 1171, 1133, 1091, 1030, 955, 889, 727, 699.

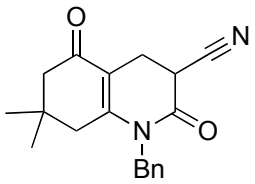


2,5-Dioxo-1-phenyl-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitrile (51b). After chromatographic purification, a yellow solid was obtained (65%, mp: 158 - 160 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.57 - 7.46 (m, 3H), 7.15 (m, 2H), 3.83 (dd, *J* = 6.4, 11.1 Hz, 1H), 3.24 (dd, *J* = 6.3, 15.2 Hz, 1H), 2.97 (m, 1H), 2.42 (dt, *J* = 1.9, 6.4 Hz, 2H), 2.13 (m, 2H), 1.96 (m, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.3, 162.7, 154.9, 136.1, 130.4, 129.9, 129.4, 128.5, 127.3,

115.5, 113.6, 36.0, 34.7, 27.9, 22.1, 21.6; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{16}H_{15}N_2O_2$: 267.1134, found 267.1134.

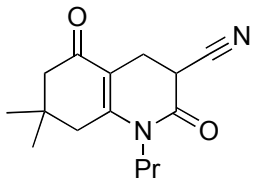


1-Butyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitrile (51c). After chromatographic purification, a colorless oil was obtained (73%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.77 (m, 1H), 3.63 (m, 2H), 3.11 (dd, *J* = 6.3, 16.5 Hz, 1H), 2.74 (m, 1H), 2.61 (m, 2H), 2.43 (t, *J* = 7.1 Hz, 2H), 2.11 (m, 2H), 1.53 (m, 2H), 1.34 (m, 2H), 0.94 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.0, 162.5, 154.4, 115.7, 114.3, 43.1, 35.8, 34.4, 31.2, 26.2, 21.8, 21.7, 20.0, 13.7; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{14}H_{19}N_2O_2$: 247.1447, found 247.1449.

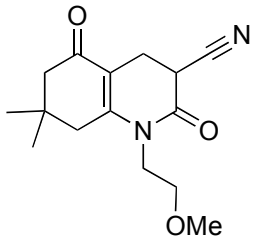


1-Benzyl-7,7-dimethyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitrile (51d). After chromatographic purification, a colorless oil was obtained (68%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.37 - 7.30 (m, 3H), 7.12 (d, *J* = 7.3 Hz, 2H), 5.13 (d, *J* = 16.4 Hz, 1H), 4.89 (d, *J* = 16.4 Hz, 1H), 3.91 (s, 1H), 3.74 (dd, *J* = 6.1, 10.0 Hz, 1H), 3.10 (dd, *J* = 6.0, 16.6 Hz, 1H), 2.96 (m, 1H), 2.39 (s, 2H), 2.26 (d, *J* = 2.2 Hz, 2H), 0.99 (s, 3H), 0.96 (s, 3H); ^{13}C NMR ($CDCl_3$,

400 MHz, ppm): δ 195.1, 163.1, 152.7, 135.8, 129.2, 127.9, 126.0, 115.6, 113.6, 49.5, 45.9, 39.9, 34.3, 33.0, 28.4, 27.8, 21.5; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{19}H_{21}N_2O_2$: 309.1603, found 309.1602.



7,7-Dimethyl-2,5-dioxo-1-propyl-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitrile (51e). After chromatographic purification, a white solid was obtained (75%, mp: 136 - 137 °C). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.67 (m, 1H), 3.57 (m, 2H), 2.98 (dd, *J* = 6.3, 16.6 Hz, 1H), 2.76 (m, 1H), 2.43 (d, *J* = 17.0 Hz, 1H), 2.36 (d, *J* = 16.9 Hz, 1H), 2.25 (d, *J* = 3.2 Hz, 2H), 1.53 (m, 2H), 1.08 (s, 3H), 1.06 (s, 3H), 0.88 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 194.0, 161.7, 151.5, 114.8, 112.2, 48.5, 43.6, 38.9, 33.4, 32.1, 27.5, 27.3, 21.5, 20.5, 10.0; ESI-HRMS: calc'd *m/e* for [M+H$^+$] $C_{15}H_{21}N_2O_2$: 261.1603, found 261.1612.



1-(2-Methoxyethyl)-7,7-dimethyl-2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinoline-3-carbonitrile (51f). After chromatographic purification, a

white solid was obtained (67%, mp: 77 - 79 °C)). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 3.91 (m, 2H), 3.63 (dd, *J* = 6.2, 11.1 Hz, 1H), 3.52 (m, 2H), 3.28 (s, 3H), 3.08 (dd, *J* = 6.2, 16.3 Hz, 1H), 2.80 (m, 1H), 2.61 (d, *J* = 17.2 Hz, 1H), 2.49 (d, *J* = 17.2 Hz, 1H), 2.28 (d, *J* = 3.0 Hz, 2H), 1.10 (s, 3H), 1.08 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz, ppm): δ 195.4, 163.1, 153.6, 115.7, 112.6, 70.3, 59.1, 49.5, 43.3, 40.2, 34.4, 33.0, 28.4, 28.3, 21.5; ESI-HRMS: calc'd *m/e* for $[M+H^+]$ $C_{15}H_{21}N_2O_3$: 277.1552, found 277.1558.

3.4 References

1 United Nations Population Division World Contraceptive Use: Data on contraceptive use were compiled primarily from surveys based on nationally representative samples of women of reproductive age. *www.un.org/esa/population/publications/contraceptive2003/WallChart_CP2003.pdf* **2003**.

2 Singh, S., Darroch, J. E., Vlassoff, M., Nadeau, J. Adding it up: the benefits of investing in sexual and reproductive health care. *The Alan Guttmacher Institute and UNFPA* **2004**.

3 Heinemann, K., Saad, F., Wiesemes, M., White, S., Heinemann, L. Attitudes toward male fertility control: results of a multinational survey on four continents. *Hum. Reprod.* **2005**, *20*, 549-556.

4 Young, V. Withdrawal: said to be unreliable yet it remains popular. *Popul. Forum* **1981**, *7*, 16-17.

5 Cornia, P. B., Anawalt, B. D. Male hormonal contraception. *Expert. Opin. Emerg. Drugs* **2004**, *9*, 335-344.

6 Hendry, W. F. Vasectomy and vasectomy reversal. *Br. J. Urol.* **1994**, *73*, 337-44.

7 Mason, R. G., Connell, P. G., Bull, J. C. Reversal of vasectomy using a macroscopic technique: a retrospective study. *Ann. R. Coll. Surg. Engl.* **1997**, *79*, 420-422.

8 Wu, F. C. W., Farley, T. M. M., Peregoudov, A., Waites, G. M. H. WHO task force on methods for the regulation of male fertility. *Fertil. Steril.* **1996**, *65*, 626-626.

9 Wallace, E. M., Pye, S. D., Wild, S. R., Wu, F. C. W. Prostate-specific antigen and prostate gland size in men receiving exogenous testosterone for male contraception. *Int. J. Androl.* **1993**, *16*, 35-40.

10 Brady, B. M., Anderson, R. A. Advances in male contraception. *Expert. Opin. Inv. Drug.* **2002**, *11*, 333-344.

11 van der Spoel, A. C., Jeyakumar, M., Butters, T. D., Charlton, H. M., Moore, H. D., Dwek, R. A., Platt, F. M. Reversible infertility in male mice after oral administration of alkylated imino sugars: A nonhormonal approach to male contraception. *Proc. Natl. Acad. Sci. USA.* **2002**, *99*, 17173-17178.

12 Paulsen, H. Carbohydrates containing nitrogen or sulfur in the 'hemiacetal' ring. *Angew. Chem. Int. Edit.* **1966**, *5*, 495-510.

13 Inouye, S., Tsuruoka, T., Niida, T. Structure and synthesis of nojirimycin. *Tetrahedron* **1966**, *24*, 2125-2144.

14 Bollen, M., Stalmans, W. The antiglycogenolytic action of 1-deoxynojirimycin results from a specific inhibition of the alpha-1,6-glucosidase activity of the debranching enzyme. *Eur. J. Biochem.* **1989**, *181*, 775-780.

15 Butters, T. D., Dwek, R. A., Platt, F. M. Therapeutic applications of imino sugars in lysosomal storage disorders. *Curr. Top. Med. Chem.* **2003**, *3*, 561-561.

16 Dwek, R. A., Butters, T. D., Platt, F. M., Zitzmann, N. Targeting glycosylation as a therapeutic approach. *Nat. Rev. Drug. Discov.* **2002**, *1*, 65.

17 Platt, F. M., Neises, G. R., Dwek, R. A., Butters, T. D. N-butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. *J. Biol. Chem.* **1994**, *269*, 8362-8365.

18 Ritter, G., Krause, W., Geyer, R., Stirm, S., Wiegandt, H. Glycosphingolipid composition of human semen. *Arch. Biochem. Biophys.* **1987**, *257*, 370-378.

19 Honke, K., Hirahara, Y., Dupree, J., Suzuki, K. Paranodal junction formation and spermatogenesis require sulfoglycolipids. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4227-4232.

20 Takamiya, K., Yamamoto, A., Furukawa, K., Zhao, J. Complex gangliosides are essential in spermatogenesis of mice: Possible roles in the transport of testosterone. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12147-12152.

21 Fujimoto, H., Tadano-Aritomi, K., Tokumasu, A., Ito, K. Requirement of seminolipid in spermatogenesis revealed by UDP-galactose: ceramide galactosyltransferase-deficient mice. *J. Biol. Chem.* **2000**, *275*, 22623-22626.

22 Amory, J. K., Muller, C. H., Page, S. T., Leifke, E. Miglustat has no apparent

effect on spermatogenesis in normal men. *Hum. Reprod.* **2007**, *22*, 702-707.

23 Bone, W., Walden, C., Fritsch, M., Voigtmann, U. The sensitivity of murine spermiogenesis to miglustat is a quantitative trait: a pharmacogenetic study. *Reprod. Biol. Endocrin.* **2007**, *5*, 1-1.

24 Butters, T. D., Dwek, R. A., Platt, F. M. Inhibition of glycosphingolipid biosynthesis: application to lysosomal storage disorder. *Chem. Rev.* **2000**, *100*, 4683-4696.

25 Mellor, H. R., Platt, F. M., Dwek, R. A., Butters, T. D. Membrane disruption and cytotoxicity of hydrophobic N-alkylated imino sugars is independent of the inhibition of protein and lipid glycosylation. *Biochem. J.* **2003**, *474*, 307-314.

26 Walden, C. M., Sandhoff, R., Chuang, C., Yildiz, Y. Accumulation of glucosylceramide in murine testis, caused by Inhibition of beta-glucosidase 2: implications for spermatogenesis. *J. Biol. Chem.* **2007**, *282*, 32655.

27 Matern, H., Heinemann, H., Legler, G., Matern, S. Purification and characterization of a microsomal bile acid beta-glucosidase from human liver. *J. Biol. Chem.* **1997**, *272*, 11261-11267.

28 Matern, H., Boermans, H., Lottspeich, F., Matern, S. Molecular cloning and expression of human bile acid beta-glucosidase. *J. Biol. Chem.* **2001**, *276*, 37929-37933.

29 Boot, R. G., Verhoek, M., Donker-Koopman, W., Strijland, A., van Marle, J., Overkleeft, H. S., Wennekes, T., Aerts, J. M. F. G. Identification of the non-lysosomal glucosylceramidase as beta-glucosidase 2. *J. Biol. Chem.* **2007**,

282, 1305-1312.

30 Yildiz, Y., Matern, H., Thompson, B., Allegood, J. C. Mutation of beta-glucosidase 2 causes glycolipid storage disease and impaired male fertility. *J. Clin. Invest.* **2006**, *116*, 2985-2994.

31 Overkleeft, H. S., Renkema, G. H., Neele, J., Vianello, P., Hung, I. O., Strijland, A., van der Burg, A. M., Koomen, G., Pandit, U. K., Aerts, J. M. F. G. Generation of specific deoxynojirimycin-type inhibitors of the non-lysosomal glucosylceramidase. *J. Biol. Chem.* **1998**, *273*, 26522-26527.

32 van der Spoel, A. C., Mott, R., Platt, F. M. Differential sensitivity of mouse strains to an N-alkylated imino sugar: glycosphingolipid metabolism and acrosome formation. *Pharmacogenomics* **2008**, *9*, 717-731.

33 Demarinis, R. M., Shah, D. H., Hall, R. F., Hieble, J. P., Pendleton, R. G. Alpha-adrenergic agents. Synthesis and alpha-1-agonist activity of 2-aminotetralins. *J. Med. Chem.* **1982**, *25*, 136-141.

34 Choi, S., Isaacs, A., Clements, D., Liu, D. H., Kim, H., Scott, R. W., Winkler, J. D., DeGrado, W. F. De novo design and in vivo activity of conformationally restrained antimicrobial arylamide foldamers. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 6968-6973.

35 Collins, I., Caldwell, J. J., Fonseca, T., Donald, A., Bavetsias, V., Hunter, L. K. Structure-based design of isoquinoline-5-sulfonamide inhibitors of protein kinase B. *Bioorgan. Med. Chem.* **2006**, *14*, 1255-1273.

36 Caldwell, J. J., Davies, T. G., Donald, A., McHardy, T., Rowlands, M. G., Aherne, G. W., Hunter, L. K., Taylor, K., Ruddle, R., Raynaud, F. I., Verdonk,

M., Workman, P., Garrett, M. D., Collins, I. Identification of 4-(4-aminopiperidin-1-yl)-7H-pyrrolo[2,3-d]pyrimidines as selective inhibitors of protein kinase B through fragment elaboration. *J. Med. Chem.* **2008**, *51*, 2147-2157.

37 Matos, C. R. R., Lopes, R. S. C., Lopes, C. C. Synthesis of 1-deoxynojirimycin and N-butyl-1-deoxynojirimycin. *Synthesis* **1999**, *4*, 571-573.

38 Noguchi, H., Aoyama, T., Shioiri, T. Total synthesis and absolute configuration of radiosumin, a strong trypsin inhibitor from the blue-green alga Plectonema radiosum. *Heterocycles* **2002**, *58*, 471-504.

39 Lipshutz, B. H., Wilhelm, R. S., Kozlowski, J. A., Parker, D. Substitution reactions of secondary halides and epoxides with higher order, mixed organocuprates, R2Cu(CN)Li2: synthetic, stereochemical, and mechanistic aspects. *J. Org. Chem.* **1984**, *49*, 3928-3938.

40 Chambers, D. J., Evans, G. R., Fairbanks, A. J. An approach to the synthesis of [alpha]-(1-6)-C-disaccharides by tandem Tebbe methylenation and Claisen rearrangement. *Tetrahedron* **2005**, *61*, 7184-7192.

41 Bernet, B., Vasella, A. Carbocyclische verbindungen aus monosacchariden. I. Umsetzungen in der glucosereihe. *Helv. Chim. Acta.* **1979**, *62*, 1990-2016.

42 Kleban, M., Kautz, U., Greul, J., Hilgers, P., Kugler, R., Dong, H. Vitamin B12 catalysis of zinc-mediated 6-deoxy-6-iodopyranoside fragmentation: a mild and convenient preparation of unsaturated hexose derivatives (5-hexenoses). *Synthesis* **2000**, 1027-1033.

43 Moutel, S., Shipman, M. Stereoselective intramolecular oxime olefin cycloadditions involving carbohydrate derived precursors. *Synlett* **1998**, *12*, 1333-1334.

44 Dransfield, P. J., Moutel, S., Shipman, M., Sik, V. Stereocontrolled synthesis of polyhydroxylated hexahydro-1H-cyclopent[c]isoxazoles by intramolecular oxime olefin cycloadditions: an approach to aminocyclopentitols. *J. Chem. Soc. Perk. T 1.* **1999**, *22*, 3349-3355.

45 Whisler, M. C., Beak, P. Synthetic applications of lithiated N-Boc allylic amines as asymmetric homoenolate equivalents. *J. Org. Chem.* **2003**, *68*, 1207-1215.

46 Philips, K. D., Zemlicka, J., Horwitz, J. P. Unsaturated sugars I. Decarboxylative elimination of methyl 2,3-di-O-benzyl-[alpha]-D-glucopyranosiduronic acid to methyl 2,3-di-O-benzyl-4-deoxy-[beta]-L-threo-Pent-4-enopyranoside. *Carbohydr. Res.* **1973**, *30*, 281-286.

47 Schon, I. Sodium-liquid ammonia reduction in peptide chemistry. *Chem. Rev.* **1984**, *84*, 287-297.

48 Cox, T., Lachmann, R., Hollak, C., Aerts, J. M. F. G., van Weely, S., Hrebícek, M., Platt, F. M. Novel oral treatment of Gaucher's disease with N-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. *Lancet* **2000**, *355*, 1481-1485.

49 Turunen, B. J., Georg, G. I. Amino acid-derived enaminones: a study in ring formation providing valuable asymmetric synthons. *J. Am. Chem. Soc.* **2006**, *128*, 8702-8703.

50 Comins, D. L., Joseph, S. P., Goehring, R. R. Asymmetric synthesis of 2-alkyl(aryl)-2,3-dihydro-4-pyridones by addition of grignard reagents to chiral 1-acyl-4-methoxypyridinium salts. *J. Am. Chem. Soc.* **1994**, *116*, 4719-4728.

51 Comins, D. L. Asymmetric synthesis and synthetic utility of 2,3-dihydro-4-pyridones. *J. Heterocyclic. Chem.* **1999**, *36*, 1491-1500.

52 Linder, M. R., Steure, S., Podlech, J. (S)-3-(tert-butyloxycarbonylamino)-4-phenylbutanoic acid. *Org. Synth.* **2003**, *79*, 154-164.

53 Vasanthakumar, G., Patil, B. S., Babu, V. V. S. Homologation of [alpha]-amino acids to [beta]-amino acids using Boc2O. *J. Chem. Soc. Perk. T 1.* **2002**, 2087-2089.

54 Nahm, S., Weinreb, S. M. N-methoxy-n-methylamides as effective acylating agents. *Tetrahedron Lett.* **1981**, *22*, 3815-3818.

55 Badorrey, R., Cativiela, C., Díaz-de-Villegas, M. D., Gálvez, J. A. Highly convergent stereoselective synthesis of chiral key intermediates in the synthesis of Palinavir from imines derived from -glyceraldehyde. *Tetrahedron* **2002**, *58*, 341-354.

56 Comins, D. L., Stolze, D. A., Thakker, F., McArdle, C. L. C-3 acetoxylation of N-acyl-2,3-dihydro-4-pyridones. *Tetrahedron Lett.* **1998**, *39*, 5693-5696.

57 Rubottom, G. M., Vazquez, M. A., Pelegrina, D. R. Peracid oxidation of trimethylsilyl enol ethers: A facile [alpha]-hydroxylation procedure. *Tetrahedron Lett.* **1974**, *15*, 4319-4322.

58 Hassner, A., Reuss, R. H., Pinnick, H. W. Synthetic methods. VIII. Hydroxylation of carbonyl compounds via silyl enol ethers. *J. Org. Chem.*

1975, *40*, 3427-3429.

59 Falb, E., Bechor, Y., Nudelman, A., Hassner, A., Albeck, A., Gottlieb, H. E. IOOC route to substituted chiral pyrrolidines. potential glycosidase inhibitors. *J. Org. Chem.* **1998**, *64*, 498-506.

60 Moisan, L., Thuéry, P., Nicolas, M., Doris, E., Rousseau, B. Formal synthesis of (+)-catharanthine13. *Angew. Chem. Int. Ed.* **2006**, *45*, 5334-5336.

61 Scholl, M., Ding, S., Lee, C. W., Grubbs, R. H. Synthesis and activity of a new generation of ruthenium-based olefin metathesis catalysts coordinated with 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene ligands. *Org. Lett.* **1999**, *1*, 953-956.

62 Luche, J. L. Lanthanides in organic chemistry. 1. Selective 1,2 reductions of conjugated ketones. *J. Am. Chem. Soc.* **2002**, *100*, 2226-2227.

63 Silverstein, R. M., Webster, F. X., Kiemle, D. J. Spectrometric Identification of Organic Compounds. *John Wiley & Sons* **2005**, 141.

64 Leverett, C. A., Cassidy, M. P., Padwa, A. Application of the aza-achmatowicz oxidative rearrangement for the stereoselective synthesis of the cassia and prosopis alkaloid family. *J. Org. Chem.* **2006**, *71*, 8591-8601.

65 Kennedy, A., Nelson, A., Perry, A. Methods for the synthesis of polyhydroxylated piperidines by diastereoselective dihydroxylation: Exploitation in the two-directional synthesis of aza-C-linked disaccharide derivatives. *Beilstein J. Org. Chem.* **2005**, *1*, 2-2.

66 Hoye, T. R., Hanson, P. R., Vyvyan, J. R. A practical guide to first-order multiplet analysis in 1H NMR spectroscopy. *J. Org. Chem.* **1994**, *59*, 4096-

4103.

67 Hoye, T. R., Zhao, H. A method for easily determining coupling constant values: an addendum to a practical guide to first-order multiplet analysis in 1H NMR spectroscopy. *J. Org. Chem.* **2002**, *67*, 4014-4016.

68 Ray, R., Matteson, D. S. Osmium tetroxide catalyzed hydroxylation of hindered olefins. *Tetrahedron Lett.* **1980**, *21*, 449-450.

69 Haukaas, M. H., O'Doherty, G. A. Synthesis of d- and l-deoxymannojirimycin via an asymmetric aminohydroxylation of vinylfuran. *Org. Lett.* **2001**, *3*, 401-404.

70 Sabitha, G., Sudhakar, K., Reddy, N. M., Rajkumar, M., Yadav, J. S. Chelation-controlled reduction: an enantioselective synthesis of (-)-tarchonanthuslactone.s *Tetrahedron Lett.* **2005**, *46*, 6567-6570.

71 Raifeld, Y. E. Asymmetric synthesis of 3-substituted furanoside compounds. *CAPatent* **1992**, *2068226*.

72 Gao, Y., Klunder, J. M., Hanson, R. M., Masamune, H., Ko, S. Y., Sharpless, K. B. Catalytic asymmetric epoxidation and kinetic resolution: modified procedures including in situ derivatization. *J. Am. Chem. Soc.* **2002**, *109*, 5765-5780.

73 Alegret, C., Santacana, F., Riera, A. Enantioselective synthesis of trans-4-methylpipecolic acid. *J. Org. Chem.* **2007**, *72*, 7688-7692.

74 Murruzzu, C., Riera, A. Enantioselective synthesis of hydroxylated pyrrolidines via Sharpless epoxidation and olefin metathesis. *Tetrahedron-Asymmetr.* **2007**, *18*, 149-154.

75 Chini, M., Crotti, P., Macchia, F. Regioalternating selectivity in the metal salt catalyzed aminolysis of styrene oxide. *J. Org. Chem.* **2002**, *56*, 5939-5942.

76 Chini, M., Crotti, P., Flippin, L. A., Gardelli, C., Giovani, E., Macchia, F., Pineschi, M. Regiochemical control of the ring opening of 1,2-epoxides by means of chelating processes. 5. Synthesis and reactions of some 2,3-epoxy-1-alkanol derivatives. *J. Org. Chem.* **2002**, *58*, 1221-1227.

77 Caron, M., Sharpless, K. B. Titanium isopropoxide-mediated nucleophilic openings of 2,3-epoxy alcohols. A mild procedure for regioselective ring-opening. *J. Org. Chem.* **2002**, *50*, 1557-1560.

78 Sharma, G. V. M., Pendem, N., Ravinder, R. K., Radha, K., P., Narsimulu, K., Kunwar, A. C. The stereoselective synthesis of C-linked 4'-deoxy aza-disaccharides from C-linked carbo-[beta]-amino acids. *Tetrahedron Lett.* **2004**, *45*, 8807-8810.

79 Bayler, H. *Photogenerated Reagents in Biochemistry and Molecular Biology. Elsevier. New York* **1983**.

80 Chapman, O. L., Leroux, J. P. 1-Aza-1,2,4,6-Cycloheptatetraene. *J, Am. Chem. Soc.* **1978**, *100*, 282-285.

81 Tash, J. Gamendazole: A potent antispermatogenic contraceptive agent with novel targets Hsp90 beta and eEF1A. *Biol. Reprod* **2008**, *78*, 91-91.

82 Michael, J. P., Koning, C. B., Gravestock, D., Hosken, G. D. Enaminones: versatile intermediates for natural product synthesis. *Pure Appl. Chem.* **1999**, *71*, 979.

83 Burke, M. D., Schreiber, S. L. A planning strategy for diversity-oriented

synthesis. *Angew. Chem. Int. Ed.* **2004**, *43*, 46-58.

84 Comins, D. L., Dehghani, A. Pyridine-derived triflating reagents: An improved preparation of vinyl triflates from metallo enolates. *Tetrahedron Lett.* **1992**, *33*, 6299-6302.

85 Johnson, C. R., Adams, J. P., Braun, M. P., Senanayake, C. B. W., Wovkulich, P. M., Uskokovic, M. R. Direct [alpha]-iodination of cycloalkenones. *Tetrahedron Lett.* **1992**, *33*, 917-918.

86 Kim, J. M., Na, J. E., Kim, J. N. [alpha]-Iodination of enaminones using the modified Johnson's procedure: the use of I2 and Et3N. *Tetrahedron Lett.* **2003**, *44*, 6317-6318.

87 Wang, X., Turunen, B. J., Leighty, M. W., Georg, G. I. Microwave-assisted Suzuki-Miyaura couplings on [alpha]-iodoenaminones. *Tetrahedron Lett.* **2007**, *48*, 8811-8814.

88 Cordell, G. A. The alkaloids: chemistry and biology. *Elsevier* **2003**, *60*.

89 Ge, H., Niphakis, M. J., Georg, G. I. Palladium(II)-catalyzed direct arylation of enaminones using organotrifluoroborates. *J. Am. Chem. Soc.* **2008**, *130*, 3708-3709.

90 Zhou, J., Fu, G. C. Cross-couplings of unactivated secondary alkyl halides: room-temperature nickel-catalyzed Negishi reactions of alkyl bromides and iodides. *J. Am. Chem. Soc.* **2003**, *125*, 14726-14727.

91 Comins, D. L., Hiebel, A., Huang, S. Synthesis and reactivity of N-acyl-5- (1-hydroxyalkyl)-2,3-dihydro-4-pyridones. *Org. Lett.* **2001**, *3*, 769-771.

92 Mannich, C. Synthesis of beta-ketonic bases. *J. Chem. Soc. Abstracts* **1917**,

112, 634.

93 Mannich, C. Synthesis of beta-ketonic bases. *Arch. Pharm.* **1917**, *255*, 261-276.

94 Bensel, N., Pevere, V., Desmurs, J. R., Wagner, A., Mioskowski, C. Straightforward synthesis of N-protected benzylic amines by carbamoalkylation of aromatic compounds. *Tetrahedron Lett.* **1999**, *40*, 879-882.

95 Saidi, M. R., Azizi, N., Naimi-Jamal, M. R. Lithium perchlorate assisted one-pot three-component aminoalkylation of electron-rich aromatic compounds. *Tetrahedron Lett.* **2001**, *42*, 8111-8113.

96 Saidi, M. R., Azizi, N., Zali-Boinee, H. A simple one-pot three-component reaction for preparation of secondary amines and amino esters mediated by lithium perchlorate.s *Tetrahedron* **2001**, *57*, 6829-6832.

97 Saidi, M. R., Mojtahedi, M. M., Bolourtchian, M. A simple three-component reaction for the preparation of silylated primary amines. *Tetrahedron Lett.* **1997**, *38*, 8071-8072.

98 Saidi, M. R., Azizi, N. A new protocol for the one-pot preparation of highly diastereomerically enriched secondary amines and [beta]-amino esters mediated by lithium perchlorate solution in diethyl ether. *Tetrahedron: Asymmetr.* **2002**, *13*, 2523-2527.

99 Naimi-Jamal, M. R., Ipaktschi, J., Saidi, M. R. The LiClO4-mediated synthesis of beta-(dialkylamino) sulfoxides and beta-(dialkylamino) sulfones by addition of lithiated salts of sulfoxides and sulfones to aldehydes and

(trimethylsilyl)dialkylamines. *Eur. J. Org. Chem.* **2000**, *9*, 1735-1739.

100 Azizi, N., Saidi, M. R. Synthesis of tertiary [alpha]-amino phosphonate by one-pot three-component coupling mediated by LPDE. *Tetrahedron* **2003**, *59*, 5329-5332.

101 Suárez, M., Ochoa, E., Verdecia, Y., Pita, B., Morán, L., Martín, N., Quinteiro, M., Seoane, C., Soto, J., Novoa, H., Blaton, N., Peters, O. M. A joint experimental and theoretical structural study of novel substituted 2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinolines. *Tetrahedron* **1999**, *55*, 875-884.

102 Tu, S., Wei, Q., Ma, H., Shi, D., Gao, Y., Cui, G. The synthesis of novel substituted 2,5-dioxo-1,2,3,4,5,6,7,8-octahydroquinolines without solvent under microwave irradation. *Synth. Commun.* **2001**, *31*, 2657.

103 Fan, X. S., Li, Y. Z., Zhang, X. Y., Qu, G. R., Wang, J. J., Hu, X. Y. An efficient and green synthesis of 1,4-dihydropyridine derivatives through multi-component reaction in ionic liquid. *Heteroat. Chem.* **2006**, *17*, 382-388.

104 Tu, S., Zhu, X., Zhang, J., Xu, J., Zhang, Y., Wang, Q., Jia, R., Jiang, B., Zhang, J., Yao, C. New potential biologically active compounds: Design and an efficient synthesis of N-substituted 4-aryl-4,6,7,8-tetrahydroquinoline-2,5(1H,3H)-diones under microwave irradiation. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2925-2928.

105 Wang, G., Miao, C. Environmentally benign one-pot multi-component approaches to the synthesis of novel unsymmetrical 4-arylacridinediones . *Green Chem.* **2006**, *8*, 1080-1085.

106 Matziari, M., Nasopoulou, M., Yiotakis, A. Active methylene phosphinic peptides: a new diversification approach. *Org. Lett.* **2006**, *8*, 2317-2319.

107 Smith, A. B., Liu, Z. Total synthesis of (-)-aplaminal. *Org. Lett.* **2008**, *10*, 4363-4365.

108 Henry, K. J., Grieco, P. A., Jagoe, C. T. Chelation controlled addition of allylstannanes to aldehydes in lithium perchlorate-diethyl ether. *Tetrahedron Lett.* **1992**, *33*, 1817-1820.

109 Tanino, K., Onuki, K., Asano, K., Miyashita, M., Nakamura, T., Takahashi, Y., Kuwajima, I. Total synthesis of ingenol. *J. Am. Chem. Soc.* **2003**, *125*, 1498-1500.

110 Lee, H., Kim, H., Yoon, T., Kim, B., Kim, S., Kim, H., Kim, D. Novel "protecting group-dependent" alkylation RCM strategy to medium-sized oxacycles: first total synthesis of (-)-isoprelaurefucin *J. Org. Chem.* **2005**, *70*, 8723-8729.

111 Gray, D., Gallagher, T. A flexible strategy for the synthesis of tri- and tetracyclic lupin alkaloids: synthesis of (+)-cytisine, (±)-anagyrine, and (±)-thermopsine13. *Angew. Chem. Int. Ed.* **2006**, *45*, 2419-2423.

112 Hourcade, S., Ferdenzi, A., Retailleau, P., Mons, S., Marazano, C. First model reactions towards the synthesis of sarain A core skeleton based upon a biogenetic scenario. *Eur. J. Org. Chem.* **2005**, *2005*, 1302-1310.

113 Barnett, C. J., Wilson, T. M., Evans, D. A., Somers, T. C. Asymmetric synthesis of dideazafolate antitumor agents via amidomethylation of nonracemic oxazolidinone imidates. Synthesis of LY309887, a cytotoxic

dideazafolate analog related to lometrexol. *Tetrahedron Lett.* **1997**, *38*, 735-738.

114 Alousi, A. A., Canter, J. M., Montenaro, M. J., J., F. D. Cardiotonic activity of milrinone, a new and potent cardiac bipyridine, on the normal and failing heart of experimental animals. *J. Cardiovasc. Pharmacol.* **1983**, *5*, 792-803.

115 Butt, E., Beltman, J., Becker, D. E., Jensen, G. S. Characterization of cyclic nucleotide phosphodiesterases with cyclic AMP analogs: topology of the catalytic sites and comparison with other cyclic AMP-binding proteins. *Mol. Pharmacol.* **1995**, *47*, 340-347.

116 Floreani, M., Fossa, P., Gessi, S., Mosti, L. New milrinone analogues: in vitro study of structure-activity relationships for positive inotropic effect, antagonism towards endogenous adenosine, and inhibition of cardiac type III phosphodiesterase. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2003**, *367*, 109-118.

117 Doering, W., Konig, E., Sturm, W. Digitalis intoxication: specificity and significance of cardiac and extracardiac symptoms. Part 1: Patients with digitalis-induced arrhythmias. *Z. Kardiol.* **1977**, *66*, 121-128.

118 Mosti, L., Schenone, P., Menozzi, G. Reaction of 2-dimethylaminomethylene-1,3-diones with dinucleophiles. V. Synthesis of 5-acyl-1,2-dihydro-2-oxo-3-pyridinecarbonitiles and 1,2,5,6,7,8-hexahydro-2,5-dioxo-3-quinolinecarboxamides. *J. Heterocyclic. Chem.* **1985**, *22*, 1503.

119 Fossa, P., Menozzi, G., Dorigo, P., Floreani, M., Mosti, L. Synthesis and pharmacological characterization of functionalized 2-pyridones structurally

related to the cardiotonic agent milrinone. *Bioorg. Med. Chem.* **2003**, *11*, 4749-4759.

120 Lo Presti, E., Boggia, R., Feltrin, A., Menozzi, G., Dorigo, P., Mosti, L. 3-Acetyl-5-acylpyridin-2(1H)-ones and 3-acetyl-7,8-dihydro-2,5(1H,6H)-quinolinediones: synthesis, cardiotonic activity and computational studies. *Il Farmaco* **1999**, *54*, 465-474.

121 Dorigo, P., Gaion, R. M., Belluco, P., Fraccarollo, D., Maragno, I., Bombieri, G., Benetollo, F., Mosti, L., Orsini, F. A pharmacological, crystallographic, and quantum chemical study of new inotropic agents. *J. Med. Chem.* **2002**, *36*, 2475-2484.

122 Lu, X., Zhang, C., Xu, Z. Reactions of electron-deficient alkynes and allenes under phosphine catalysis. *Accounts Chem. Res.* **2001**, *34*, 535-544.

123 Nair, V., Menon, R. S., Sreekanth, A. R., Abhilash, N., Biju, A. T. Engaging zwitterions in carbon-carbon and carbon-ˆ’nitrogen bond-forming reactions:a promising synthetic strategy. *Accounts Chem. Res.* **2006**, *39*, 520-530.

124 Methot, J., Roush, W. R. Nucleophilic Phosphine Organocatalysis. *Adv. Synth. Catal.* **2004**, *346*, 1035-1050.

125 Liu, H., Zhang, Q., Wang, L., Tong, X. PPh3-catalyzed [2 + 2 + 2] and [4 + 2] annulations: synthesis of highly substituted 1,2-dihydropyridines (DHPs). *Chem. Commun.* **2010**, *46*, 312-314.

126 Yadav, J. S., Subba, R. B. V., Basak, A. K., Visali, B., A., V. N., Nagaiah, K. Phosphane-catalyzed knoevenagel condensation: a facile synthesis of alpha-cyanoacrylates and alpha-cyanoacrylonitriles. *Eur. J. Org. Chem.*

2004, *3*, 546-551.

127 Yadav, J. S., Reddy, B. V. S., Krishna, A. D., Reddy, C. S., Narsaiah, A. V. Triphenylphosphine: An efficient catalyst for transesterification of [beta]-ketoesters. *J. Mol. Catal. A-Chem.* **2007**, *261*, 93-97.

128 Bieg, T., Szeja, W. Removal of *O*-benzyl protective groups by catalytic transfer hydrogenation. *Synthesis* **1985**, *75*.

129 Dermer, O. C., Edmison, M. T. Orientation in aromatic substitution by the benzenesulfonimido radical1. *J, Am. Chem. Soc.* **1955**, *77*, 70-73.